Strategy for retinal cell therapy

Patient-centered risk assesment

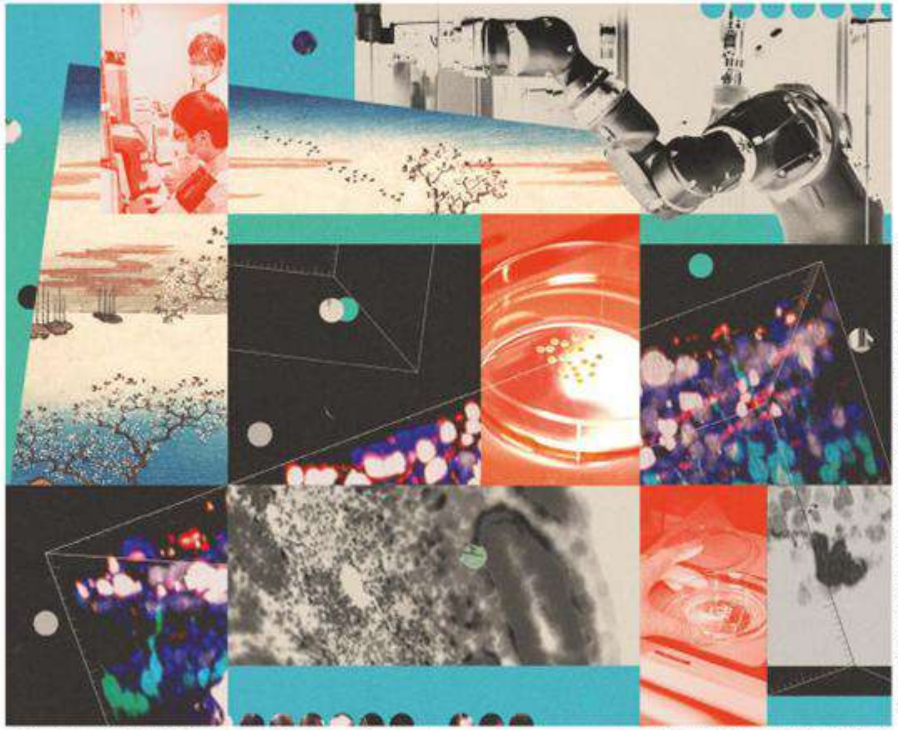
Masayo Takahashi
Vision Care group. Kobe City Eye Hospital

The first-in-man application of iPS-derived cells to treat wet AMD (2014)

Autologous iPSC-RPE transplantation

Nature's 10
Ten people who mattered
this year. 2014

Nature 2025.4.17 “JAPAN’s big bet on stem-cell therapies might soon pay off”



JAPAN’S BIG BET ON STEM-CELL THERAPIES MIGHT SOON PAY OFF

Induced pluripotent stem cells are being tested to treat blindness, paralysis, Parkinson’s disease and more. Approvals might be around the corner. **By Smriti Mallapaty**

Japan is brimming with signs of an approaching medical revolution. Shiny white robots are tending dishes of cells, rows of incubators hum in new facilities, and a deluxe, plush-carpeted hospital is getting ready to welcome its first patients.

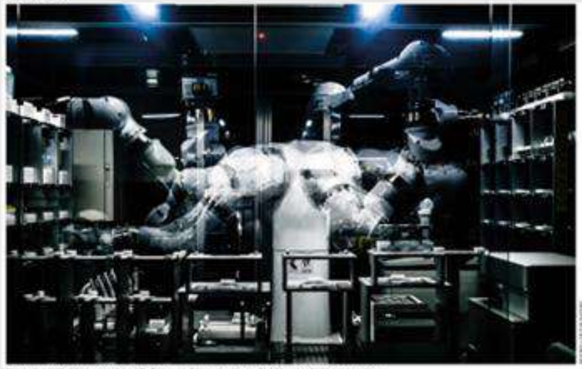
Building on the Nobel-prizewinning work of stem-cell scientist Shinya Yamanaka, researchers across the country are crafting cells into strips of retina, sheets of cardiac muscle or blobs of neurons, in the hope of treating blindness, mending hearts and reversing neurodegeneration. Results from early-stage clinical trials – some announced just in the past few weeks – suggest that the cells might actually be working to treat conditions as varied as Parkinson’s disease and spinal-cord injury.

Now, after nearly two decades of hard work and setbacks, many say that Japan is on the cusp of bringing these therapies to market.

Yamanaka, who runs a lab at Kyoto University, discovered in 2006 that adult cells could be reprogrammed into an embryonic-like state,

Masayo Takahashi ran the first clinical trials for iPS cells.

REGENERATIVE MEDICINE IN JAPAN



This robot in Kobe is preparing cells for transplant to treat people with macular degeneration.

Safety first

iPS cells for all

Medical travel

“OUR ULTIMATE GOAL IS AUTOLOGOUS TRANSPLANTATION.”

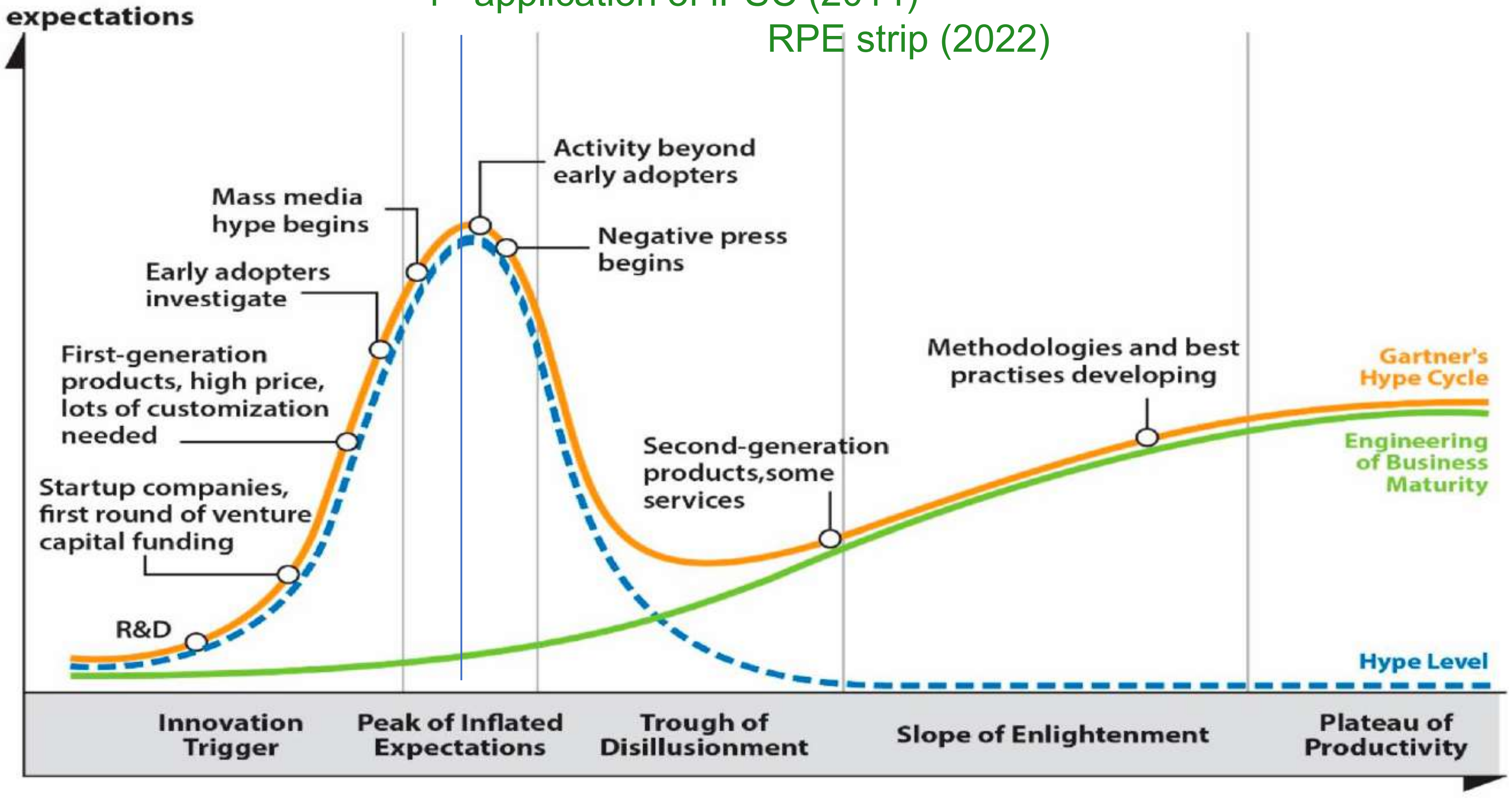
Jun Takahashi is trying to treat Parkinson’s.

Smriti Mallapaty writes for Nature from Sydney, Australia.

Gartner's hype cycle
Expectation for new technologies

The Salk Institute　Kyoto U Hp　RIKEN　VCCT

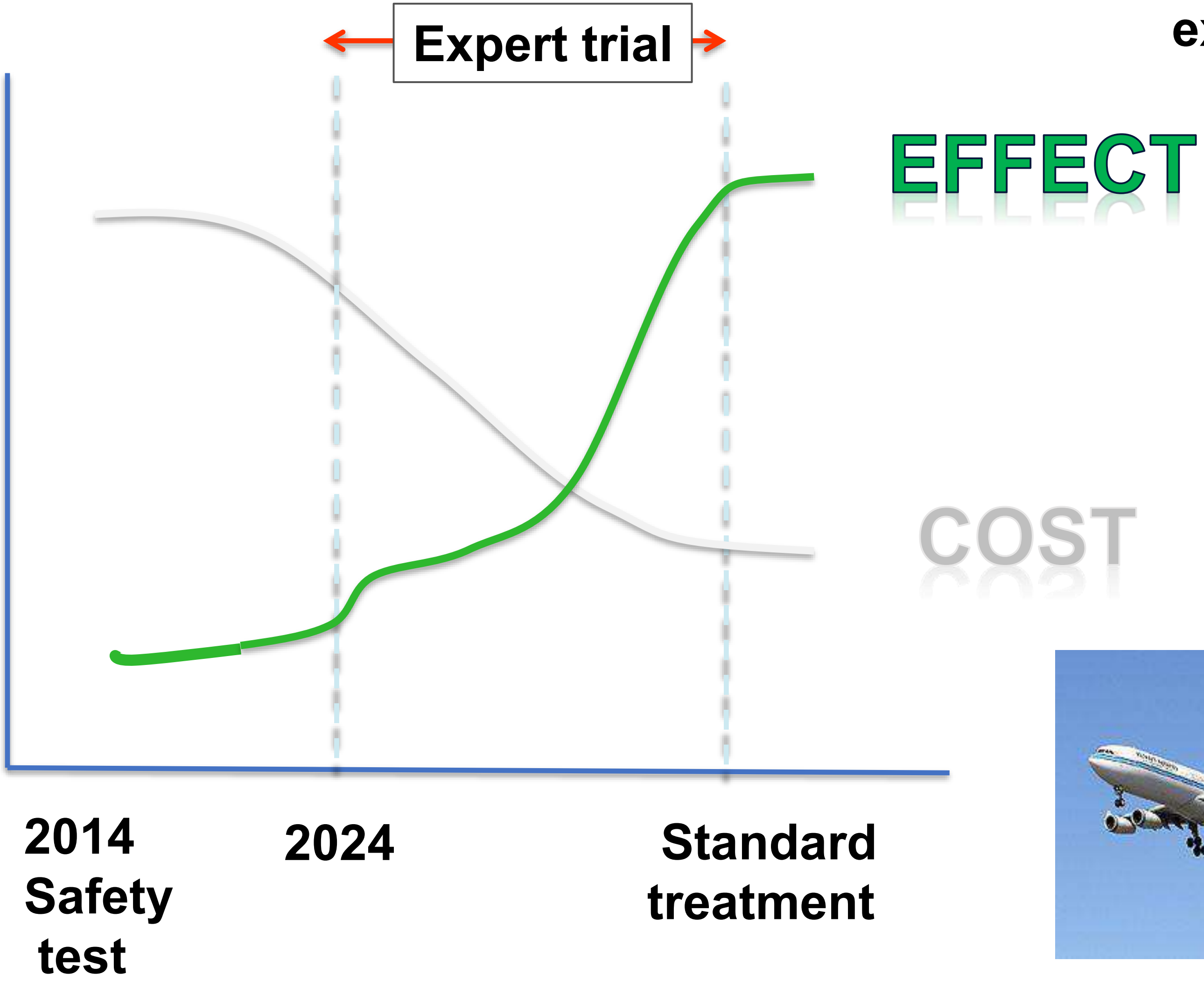
Nobel prize,
Prof.Yamanaka (2012)
1st application of iPSC (2014)
RPE strip (2022)



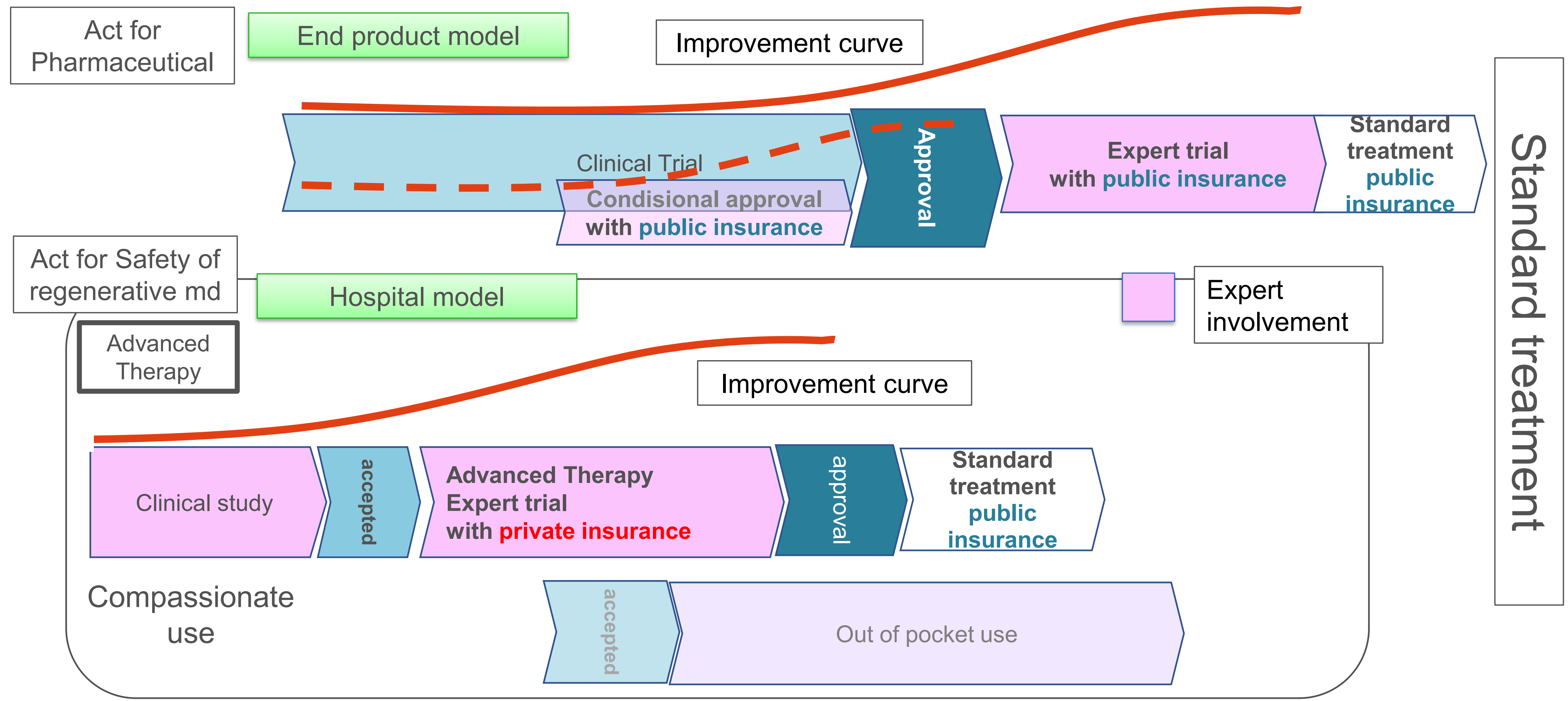
Cost vs Benefit
of regenerative medicine with **Surgery**

ex) Islet transplantation
Cataract surgery

１９０３

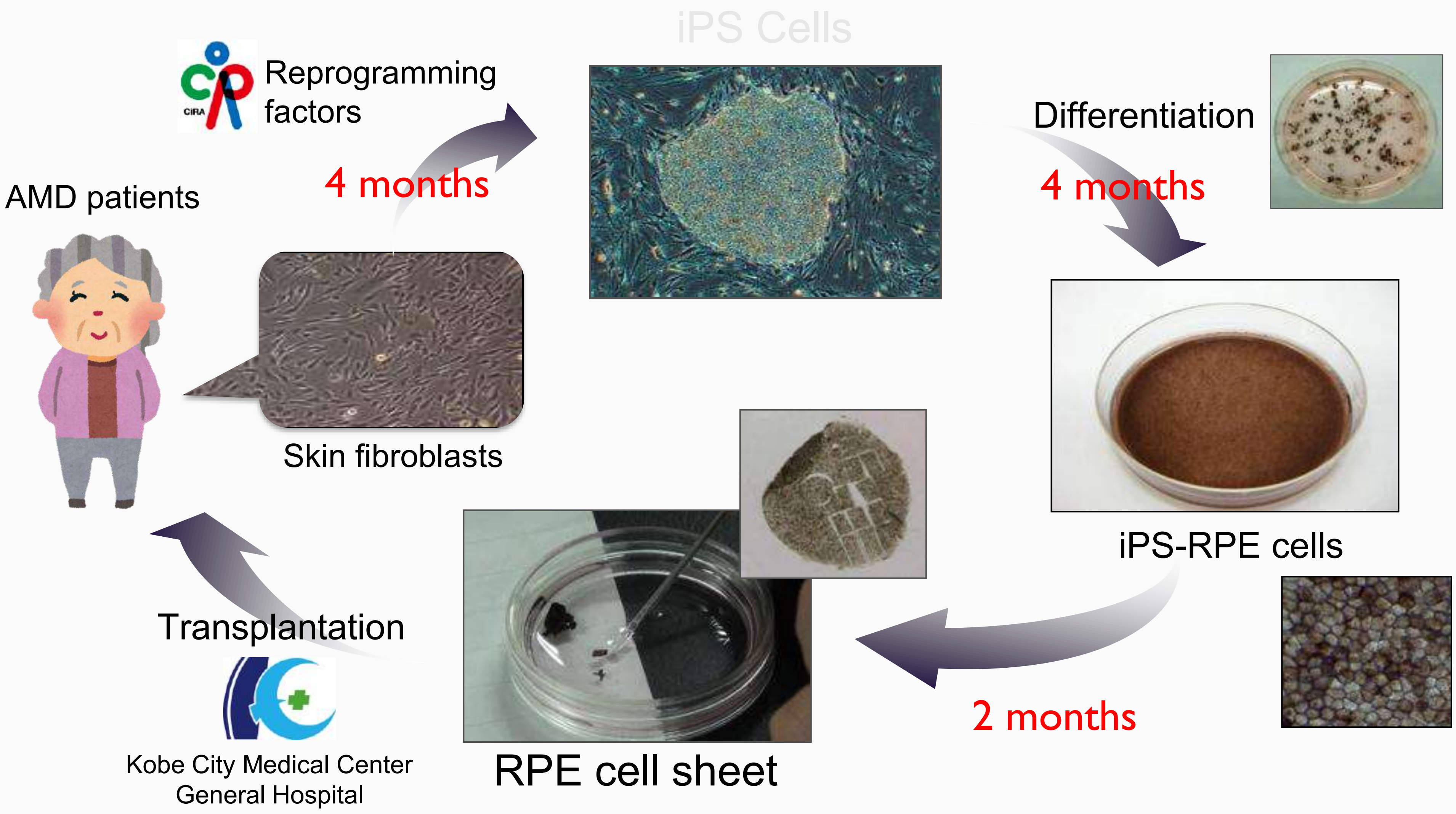


2014～ Various tracks for cell & gene therapy development in Japan



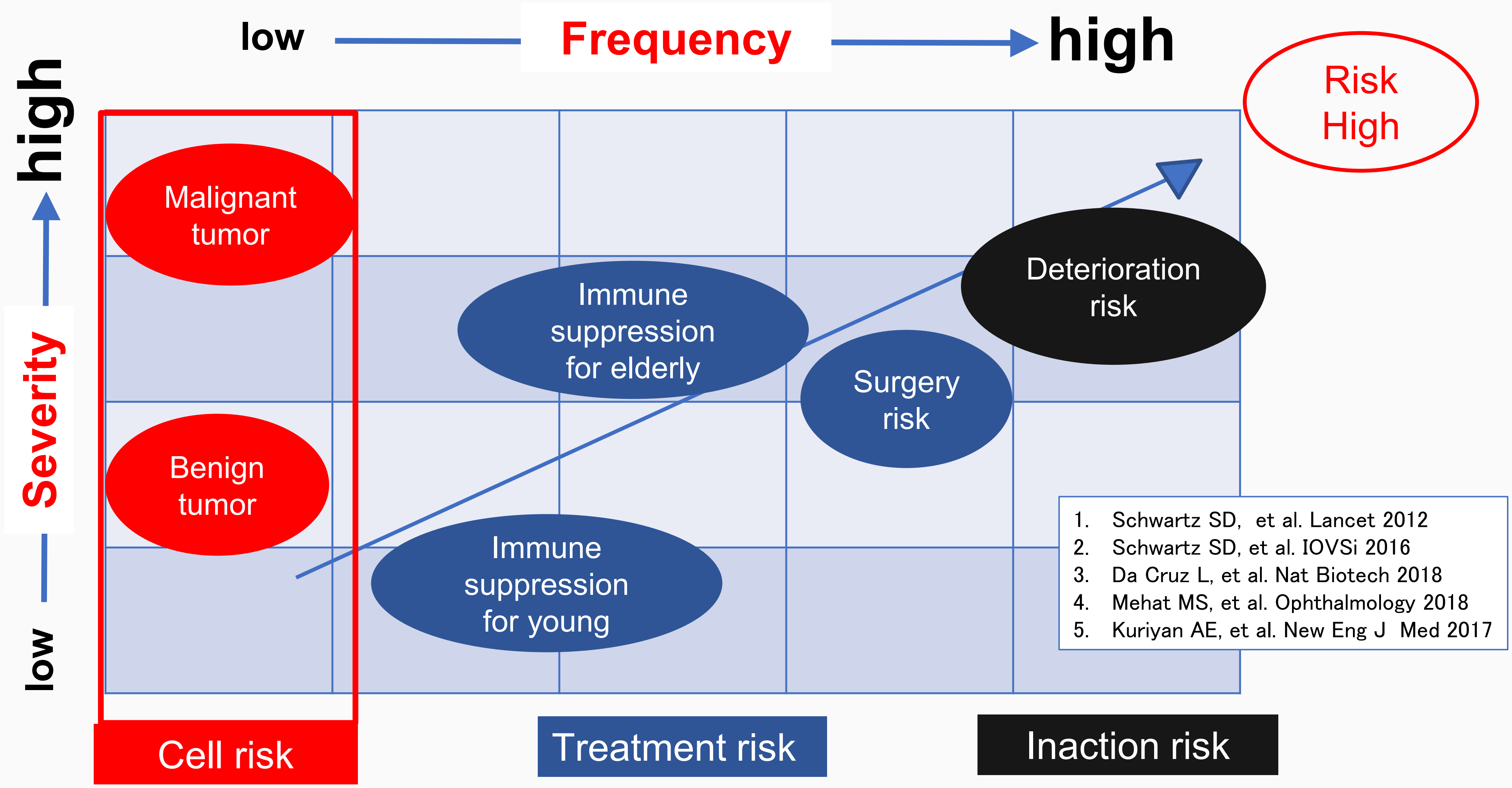
1st clnical research 2013～2015

Autologous iPS-RPE transplantation to AMD patients



Risk Matrix

for iPSC-RPE transplantation (Image)



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4. Mehat MS, et al. Ophthalmology 2018
5. Kuriyan AE, et al. New Eng J Med 2017

Strategy for RPE transplantation development

Before the 1st Case

- Determine the Mode of Action – replacement of RPE cell layer
- Determine the CQA (Critical Quality Attribute) – purity

The 2nd & 3rd clinical study

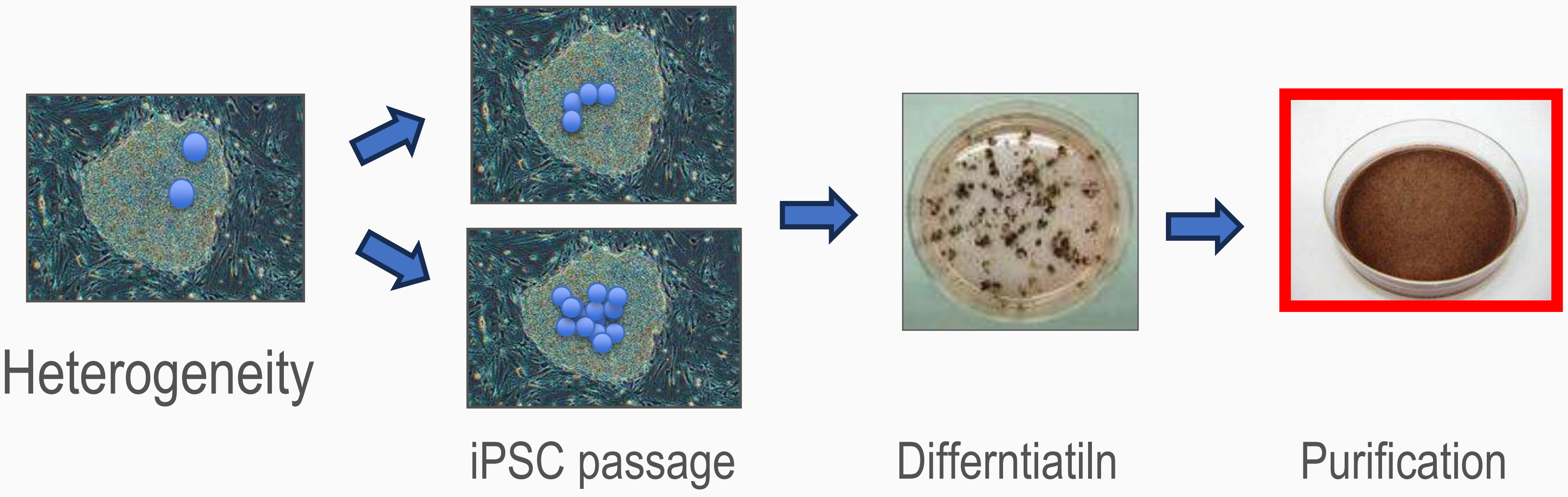
- Select the formulation to solve the risk of surgery – RPE strip
- Solve manufacturing skill transfer risks and scale up – using the humanoid robot
- Prepare to expand the surgeons and hospitals

Clinical trial

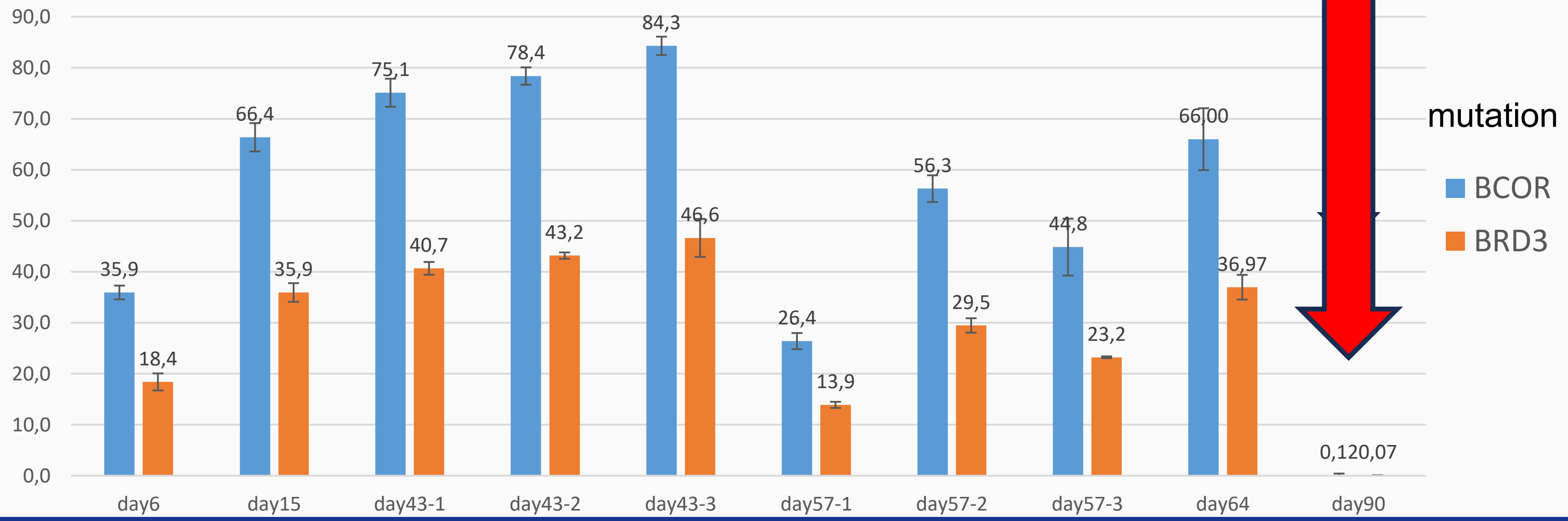
- Avoid the immune suppression risk – Modify the HLA
- Do the clinical trial with the final product

Heterogeneity of cells in the iPSC colonies

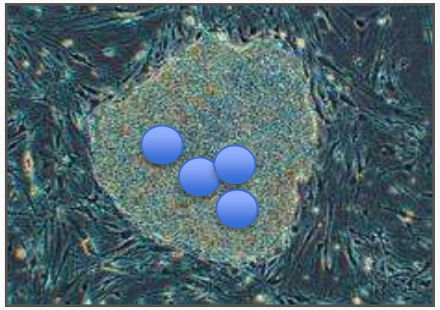
Unpublished data

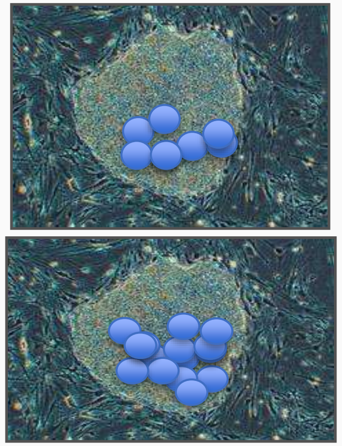


iPS-Ff- line P13 BCOR, BRD3 mutation ratio time course (n=3)



Heterogeneity between and within the iPSC colonies





Heterogeneity

MCB

X gene mutation rate
at **21.5%**

WCB — Mutation **49.3%** → RPE50% → RPE100% — Mutation rate **0.08%**

WCB — Mutation **62.8%** → RPE38% → RPE100% — Mutation rate **0.00%**

WCB — Mutation **68.5%** → RPE31% → RPE100% — Mutation rate **0.2%**

< Negative control

Gene mutation
Dominant cell alteration

Subculture

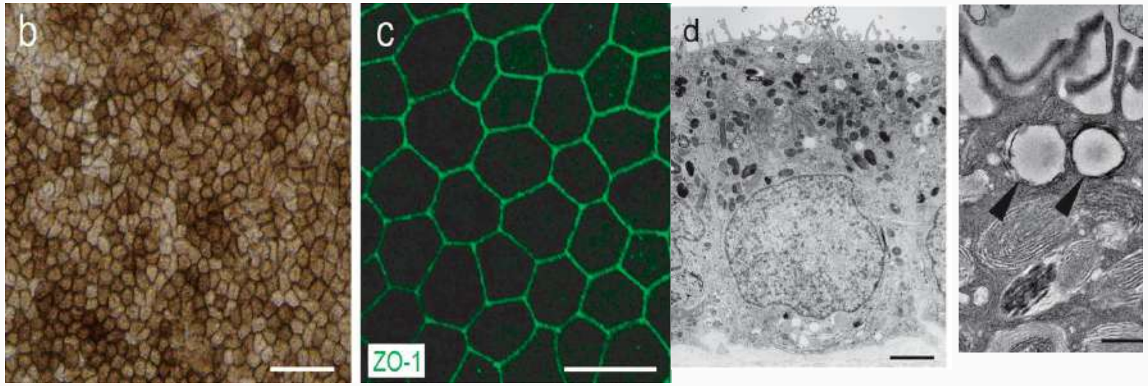
Differentiation

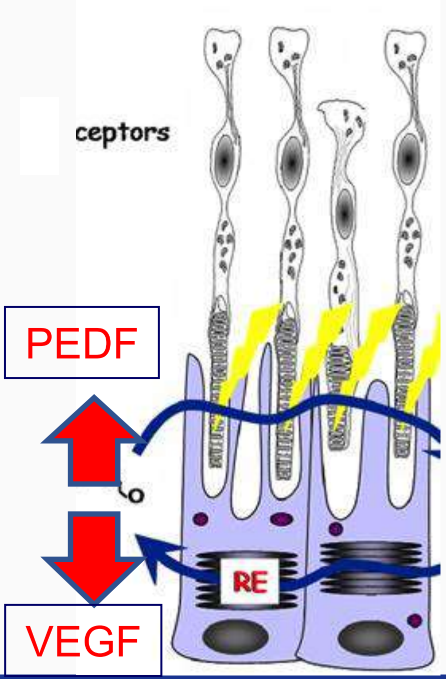
Purification

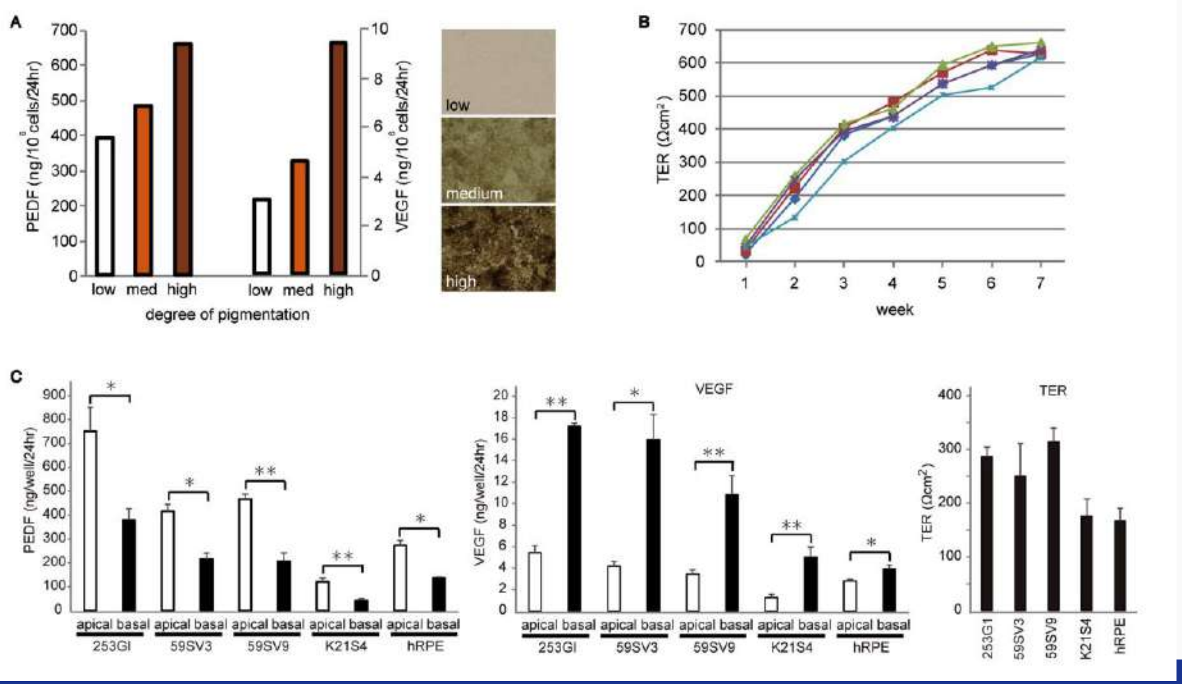
Monitoring points：RPE function

1、Phagocytosis
2、Trophic factor：
PEDF VEGF
3、Barrier formation
4、Visual cycle

Phlygonal shape　Tight junction　EM　Phago

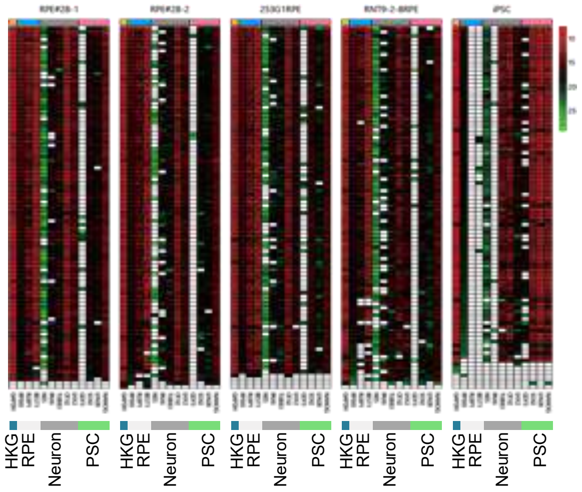






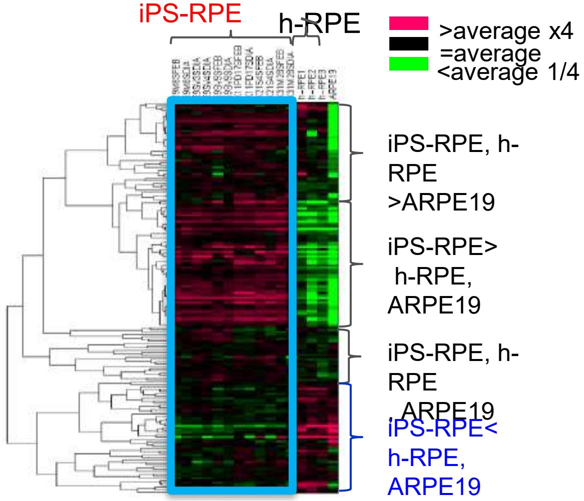
<Robust protocol >　Assessment of purity and equivalence

Single cell RT-PCR



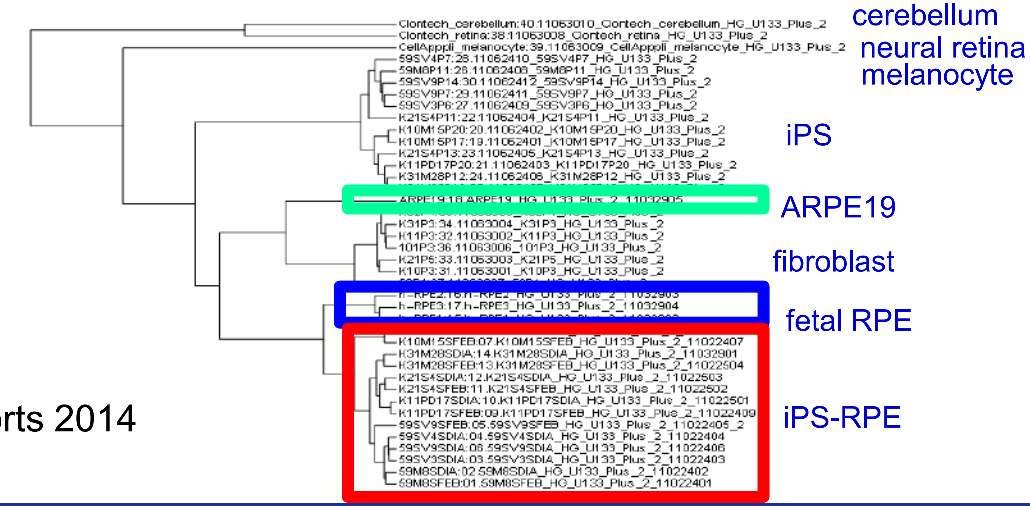
Mandai et al,
New Eng J Med, 2017

RPE signature genes



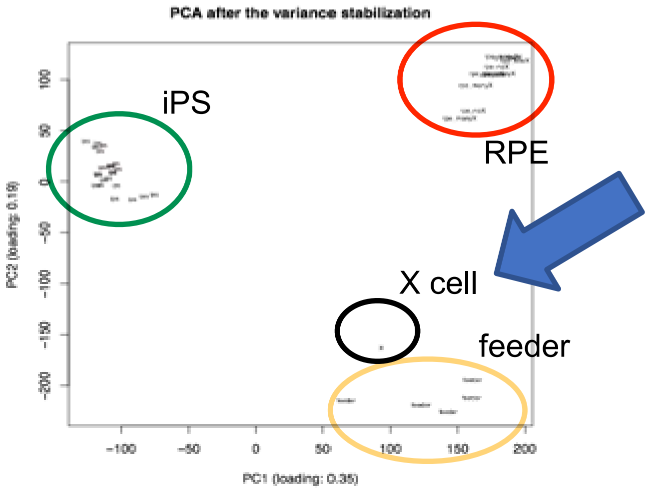
Kamao et al,
Stem Cell Reports 2014

Microarray cluster analysis



Kamao et al,
Stem Cell Reports 2014

Transcriptome analysis



Unpublished data

What are the potentially dangerous cells?

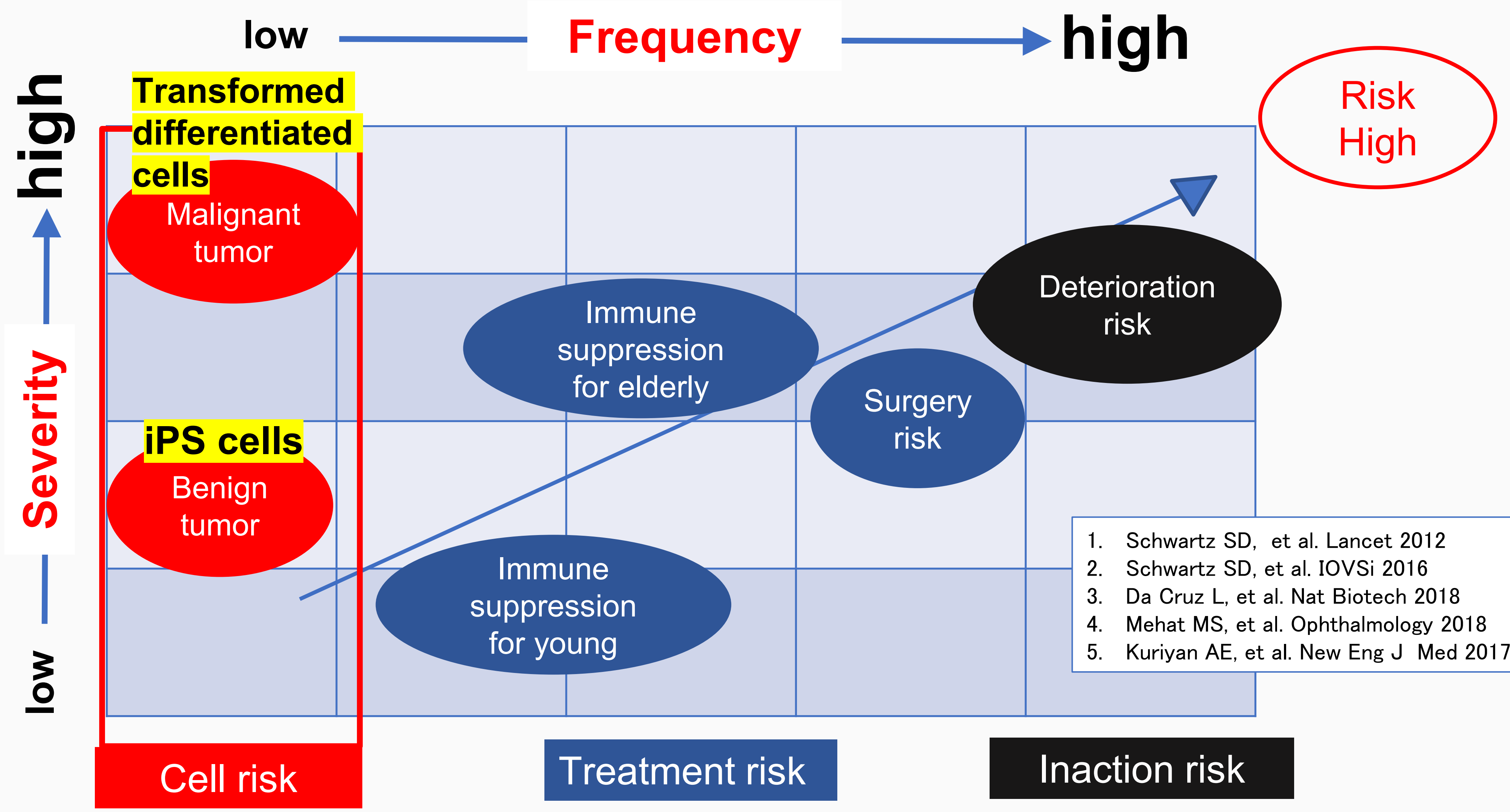
What will happen to patients?

- Residual iPS cells →benign tumor within the eye
  → typically managed with observation per Ophthalmology guidelines (no harm)
- Transformed differentiated cells → malignant tumor → metastatic tumor（life-threatening)
- Oncogene-mutated cells
  In MSCs or short –lived cells → safe
  In non dividing cells → no secondary hit → short-term tumorigenicity test (e.g., RPE)
  In dividing cells → secondary hit may occur → long-term tumorigenicity test or discontinue use

The risky cells are not iPS cells but immature differentiated or transformed cells

Risk Matrix

for iPSC-RPE transplantation (Image)



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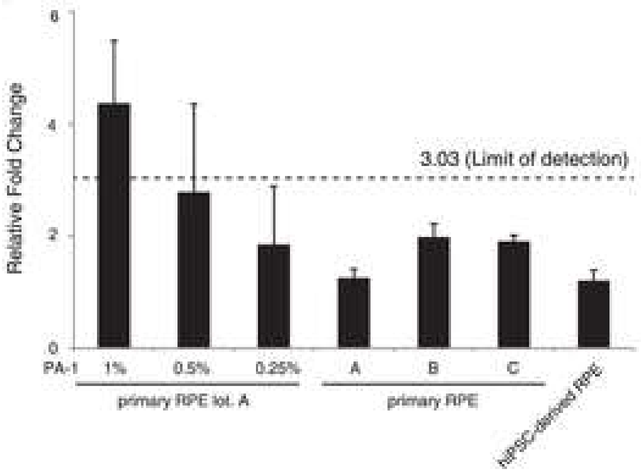
PLoS One. 2012;7(5):e37342. Epub 2012 May 17.

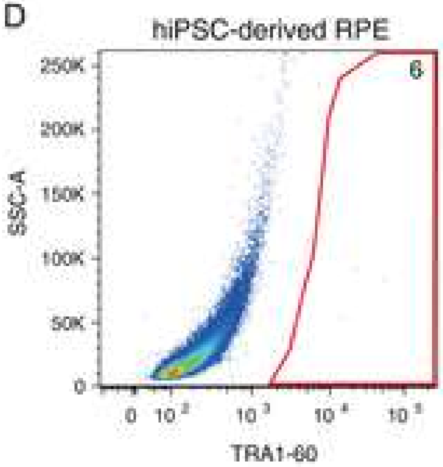
Highly Sensitive In Vitro Methods for Detection of Residual Undifferentiated Cells in Retinal Pigment Epithelial Cells Derived from Human iPS Cells.

Kuroda T, Yasuda S, Kusakawa S, Hirata N, Kanda Y, Suzuki K, Takahashi M, Nishikawa S, Kawamata S, Sato Y.
National Institute of Health Sciences, Tokyo, Japan

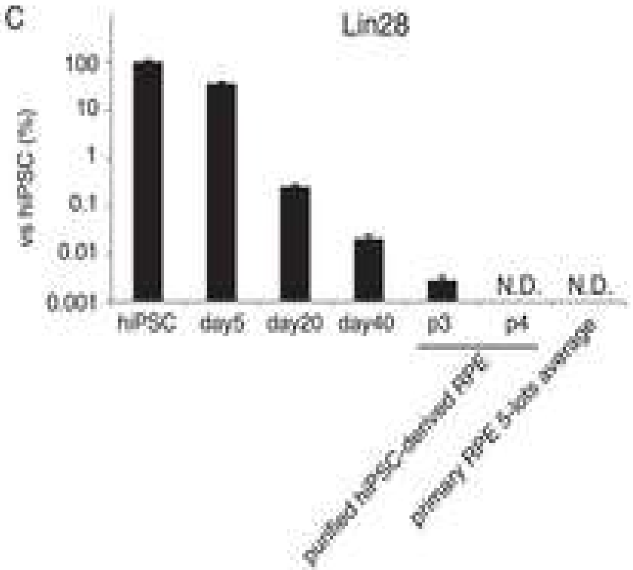
Soft Agar Test



Flowcytometry



qRT-PCR analysis



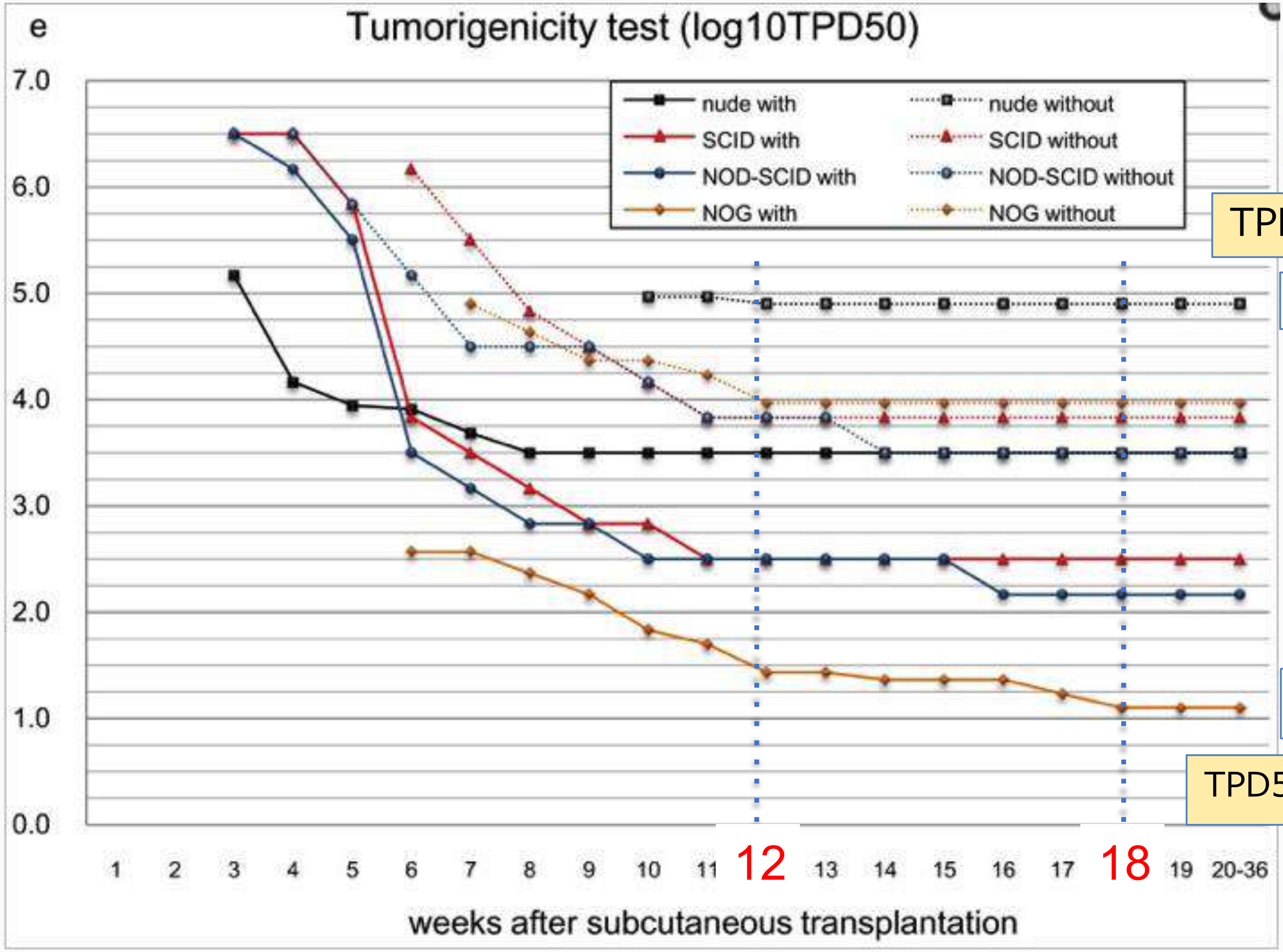
qRT-PCR analysis for Lin28 detects 0.002% of hiPSCs
a single hiPSC in 5.0×10^4 RPE cells is detectable.

Comparison among immune-deficient mice

With or without Matrigel HeLa tumorigenicity test

Kanemura H, Go MJ, Shikamura M, et al PLoS One. 9(1):e85336, 2014

Shin Kawamata



With or w/o Matrigel

TPD50=Ca 100,000 cells

Nude w/o

NOG w/o

SCID w/o

NOD w/o

nude with

SCID with

NOD with

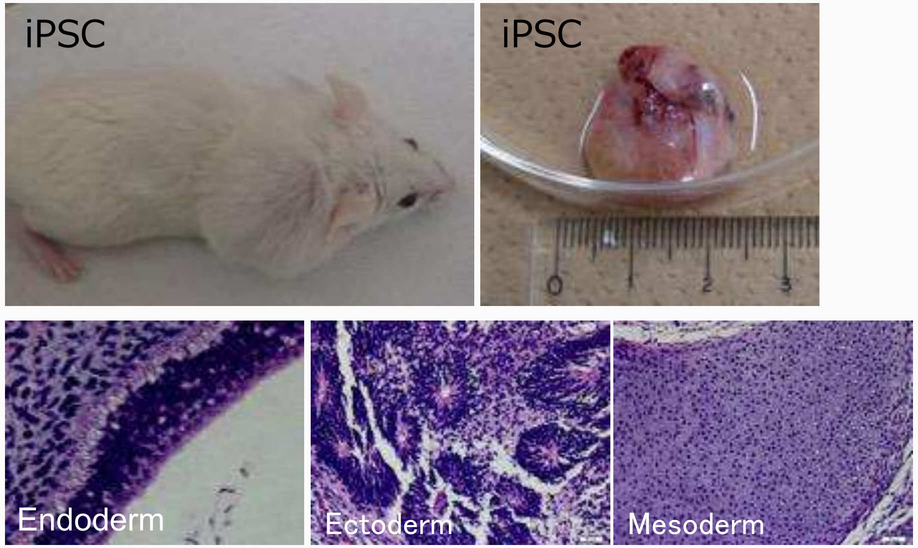
NOG with

TPD50=$10^{1.1}$ (n=75)

Kanemura et al.

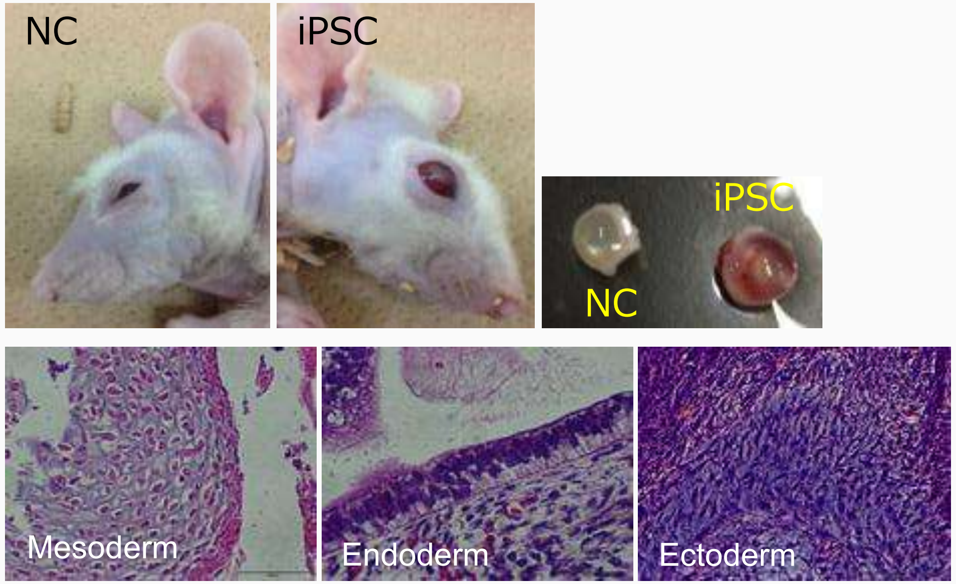
Sensitivity of tumorigenicity test (iPS Cells)

● subcutaneous (with Matrigel)- NOG mice



iPSC: TPD50= $10^{2.12}$ (n = 30)

● subretinal-(w/o Matrigel)- Nude rat



iPSC: TPD50= $10^{4.73}$ (n = 20)

Graft cells: iPSC 201B7
observation : 60w～

Summary of tumorigenicity test

（Kanemura et al.2014 PlosONE)

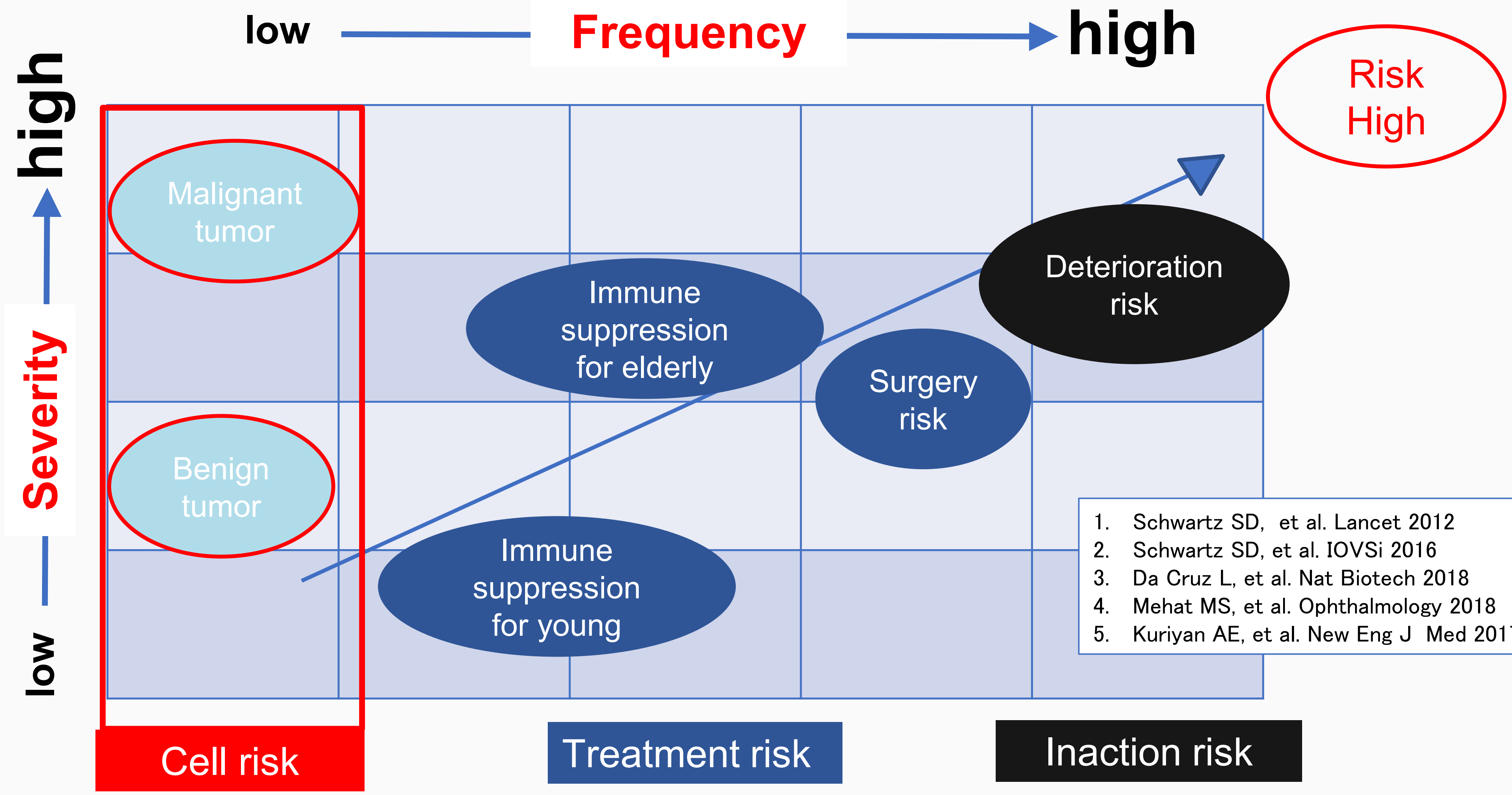
NOD-SCID mice, subcutaneous, 1 x 10＾5 cells in Matrigel

Purified hiPS−RPE did not generate tumors

	donor	Vector	# of animal	tumor
1st **2010. 11～2011. 9**	RPE cell	Retrovirus Sendai virus Plasmid A	57	**0**
2nd **2011. 2～2012 4**	RPE cell	Plasmid A	11	**0**
	RPE cell： OCT3/4 remnant+	Plasmid A	7	**0**
3rd **2012. 3～10**	RPE cell	Plasmid B	27	**0**
4th **2012. 12～2013 7**	RPE sheet （subcutaneous・subretinal）	Plasmid B	16	**0**

Risk Matrix

for iPSC-RPE transplantation (Image)



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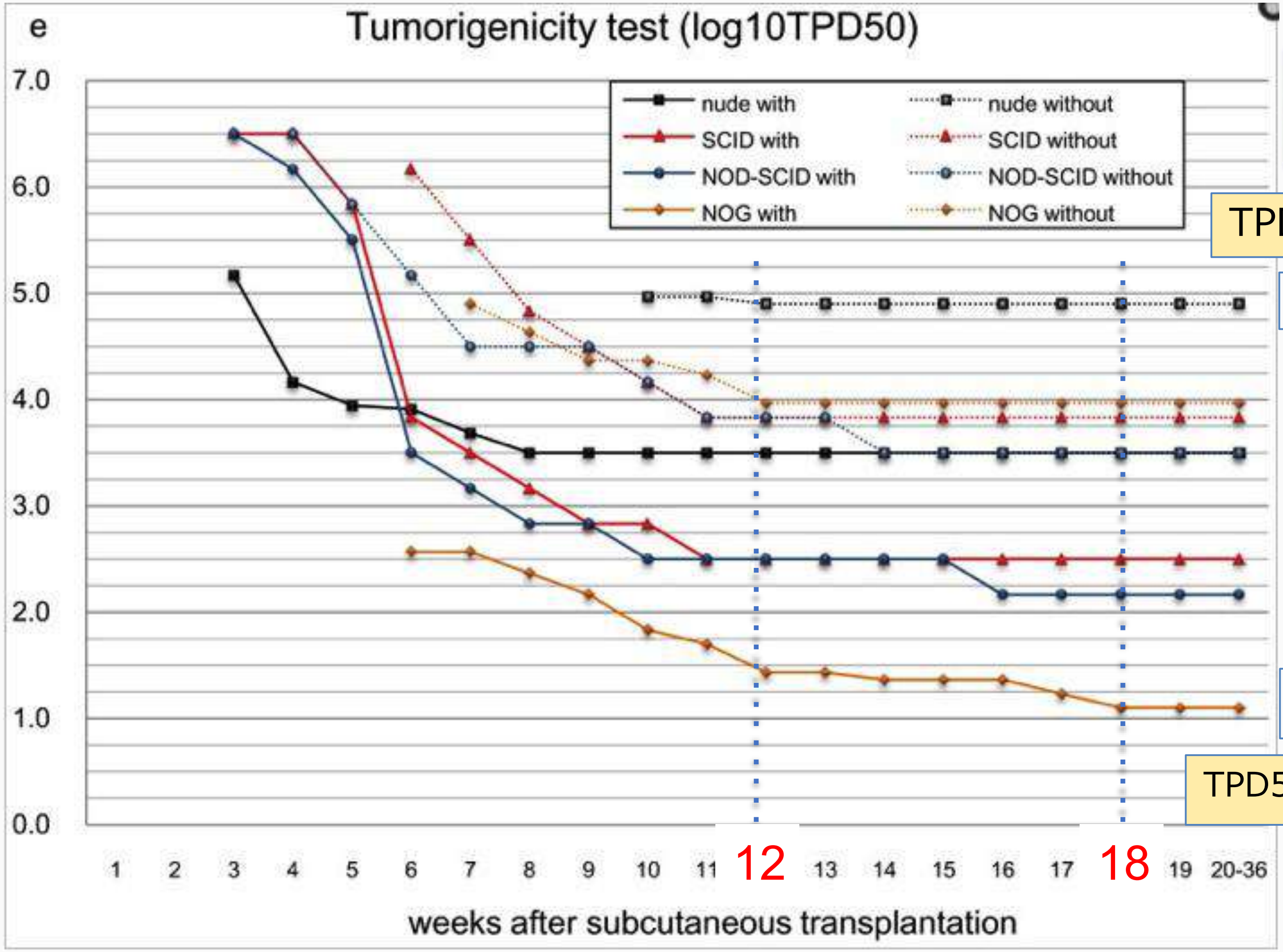

With or w/o Matrigel

TPD50=Ca 100,000 cells

Nude w/o

NOG w/o

SCID w/o

NOD w/o

nude with

SCID with

NOD with

NOG with

$TPD50=10^{1.1}$ (n=75)

Kanemura et al.

Summary of tumorigenicity test

（Kanemura et al.2014 PlosONE)

NOD-SCID mice, subcutaneous, 1 x 10 ^ 5 cells in Matrigel

Purified hiPS−RPE did not generate tumors

	donor	Vector(iPS)	# of animal	tumor
1st 2010. 11～2011. 9	RPE cell	Retrovirus Sendai virus Plasmid A	57	**0**
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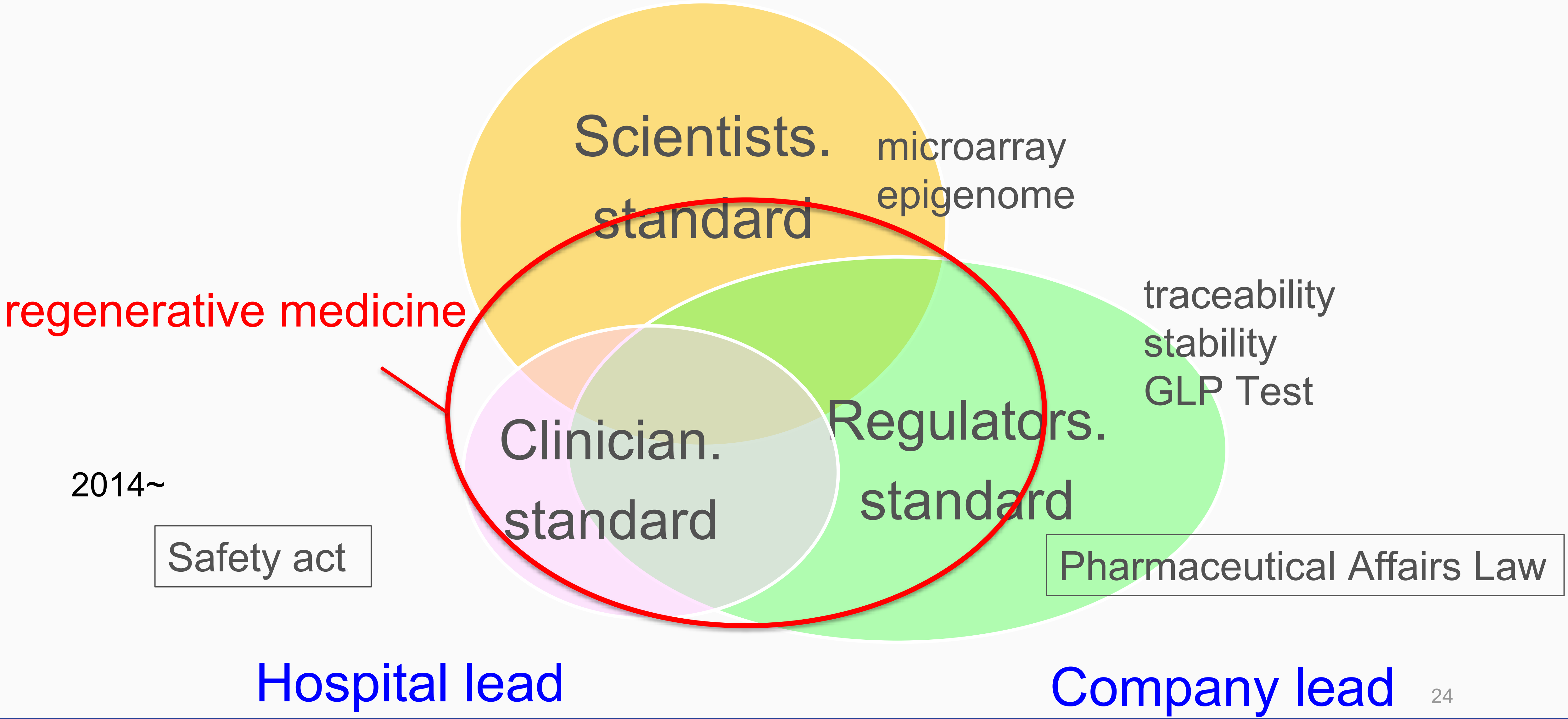
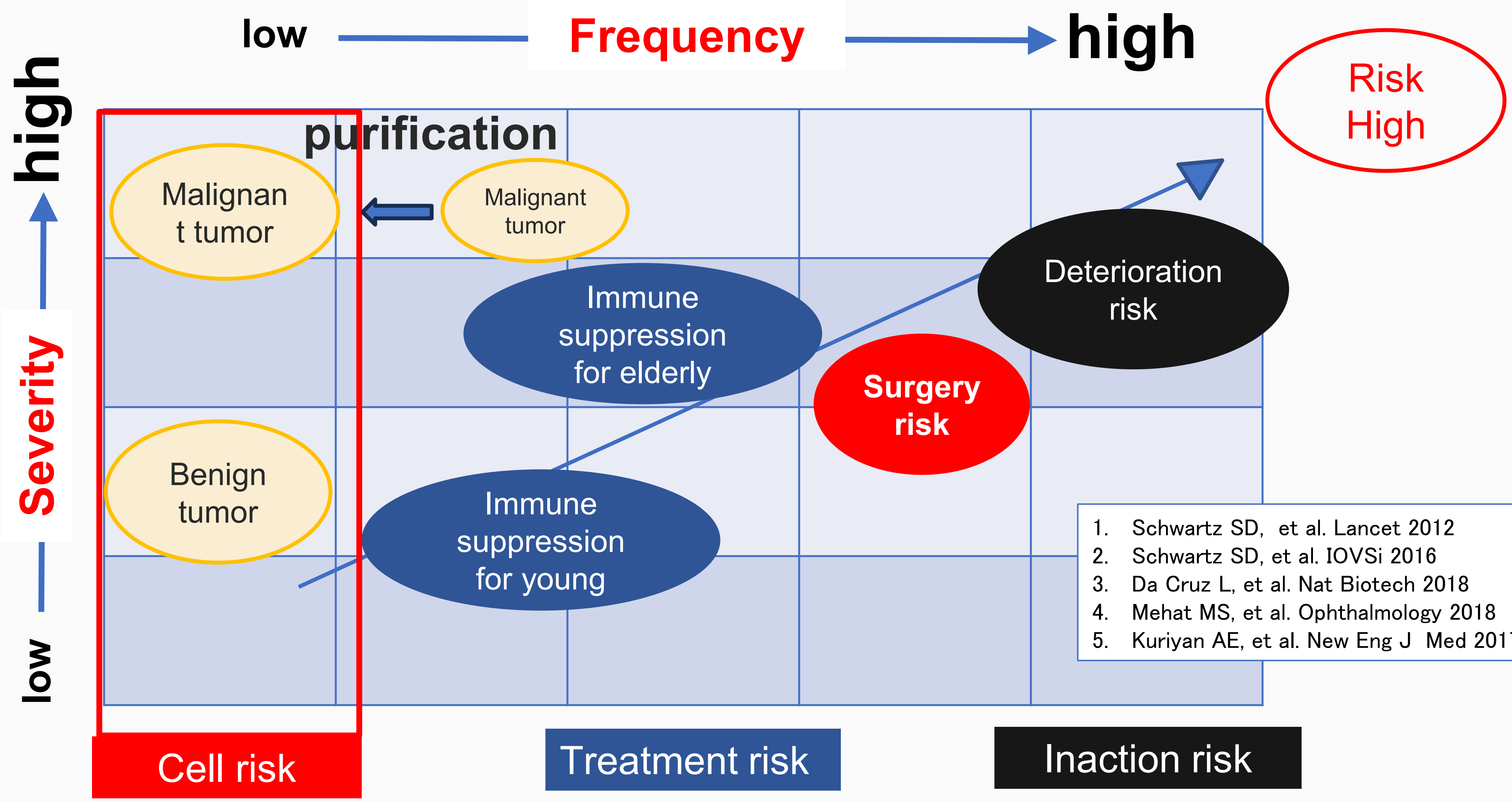
4th 2012. 12～2013 7	RPE sheet （subcutaneous・subretinal）	Plasmid B	16	**0**

Convincing data for each field



Risk Matrix

for iPSC-RPE transplantation (Image)



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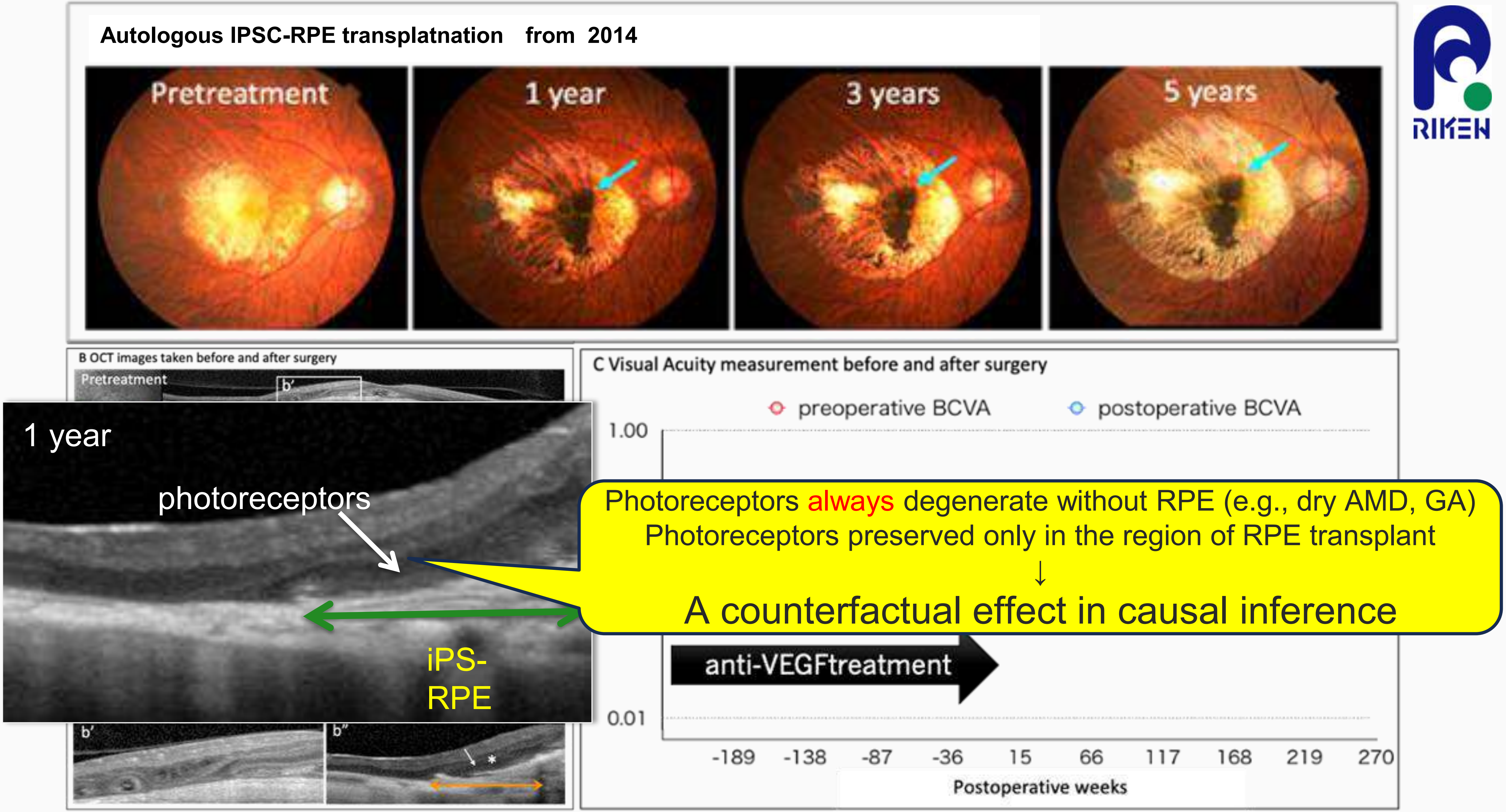
Autologous IPSC-RPE transplatnation from 2014



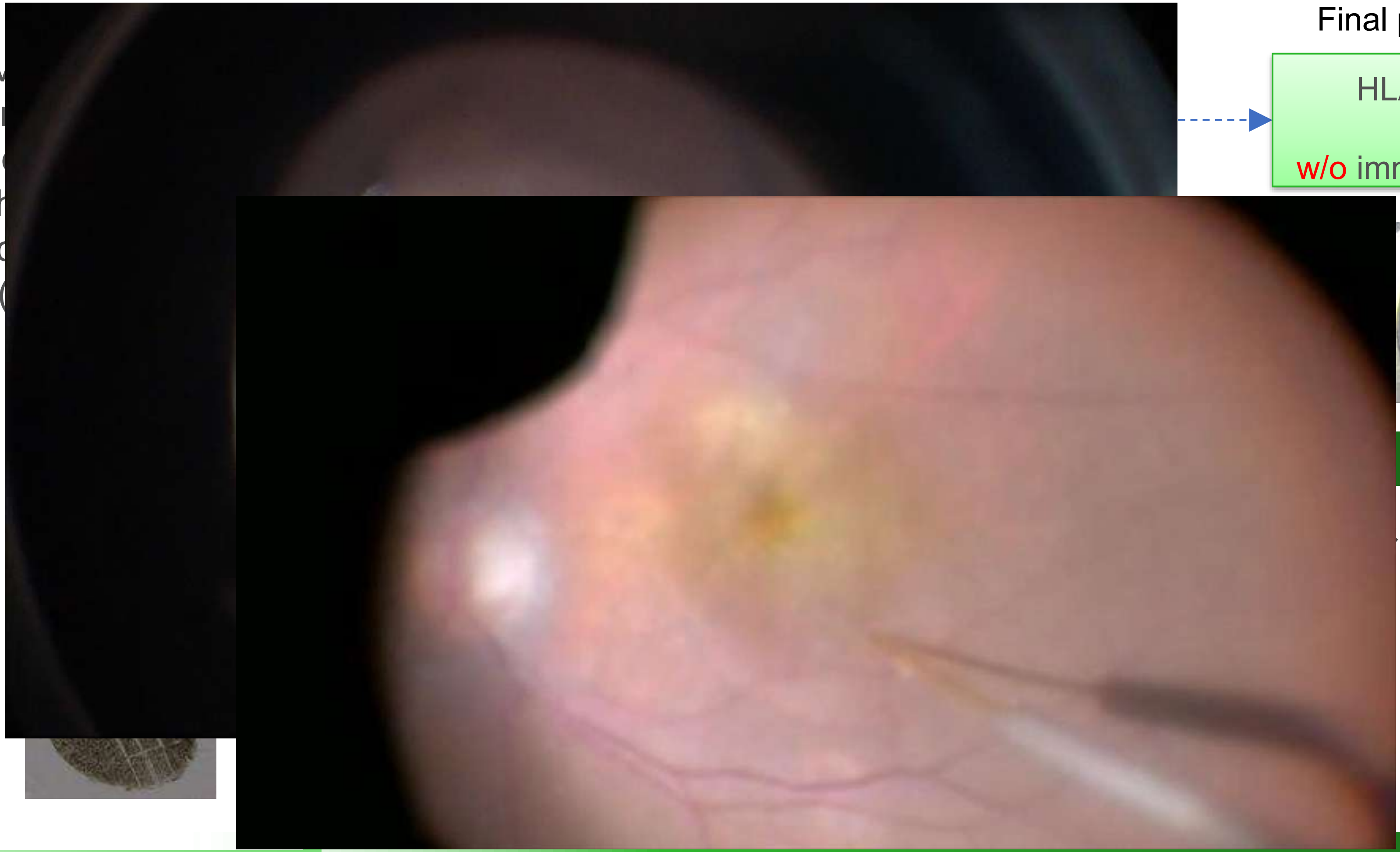
Photoreceptors always degenerate without RPE (e.g., dry AMD, GA)
Photoreceptors preserved only in the region of RPE transplant
↓
A counterfactual effect in causal inference

(Mandai et al. New Eng J Med. 2017, Takagi et al. Ophthalm Retina 2019)

RPE

For the standard treatment (2017~)
Allogeneic iPSC-RPE transplantation

Totally 10 patients
- No serious adverse ev
- No severe surgical co
- All have long survival
- All patients maintain th
- Two of them showed
subjective symptoms (



Final product

HLA partially KO
RPE strip
w/o immune suppression

RP

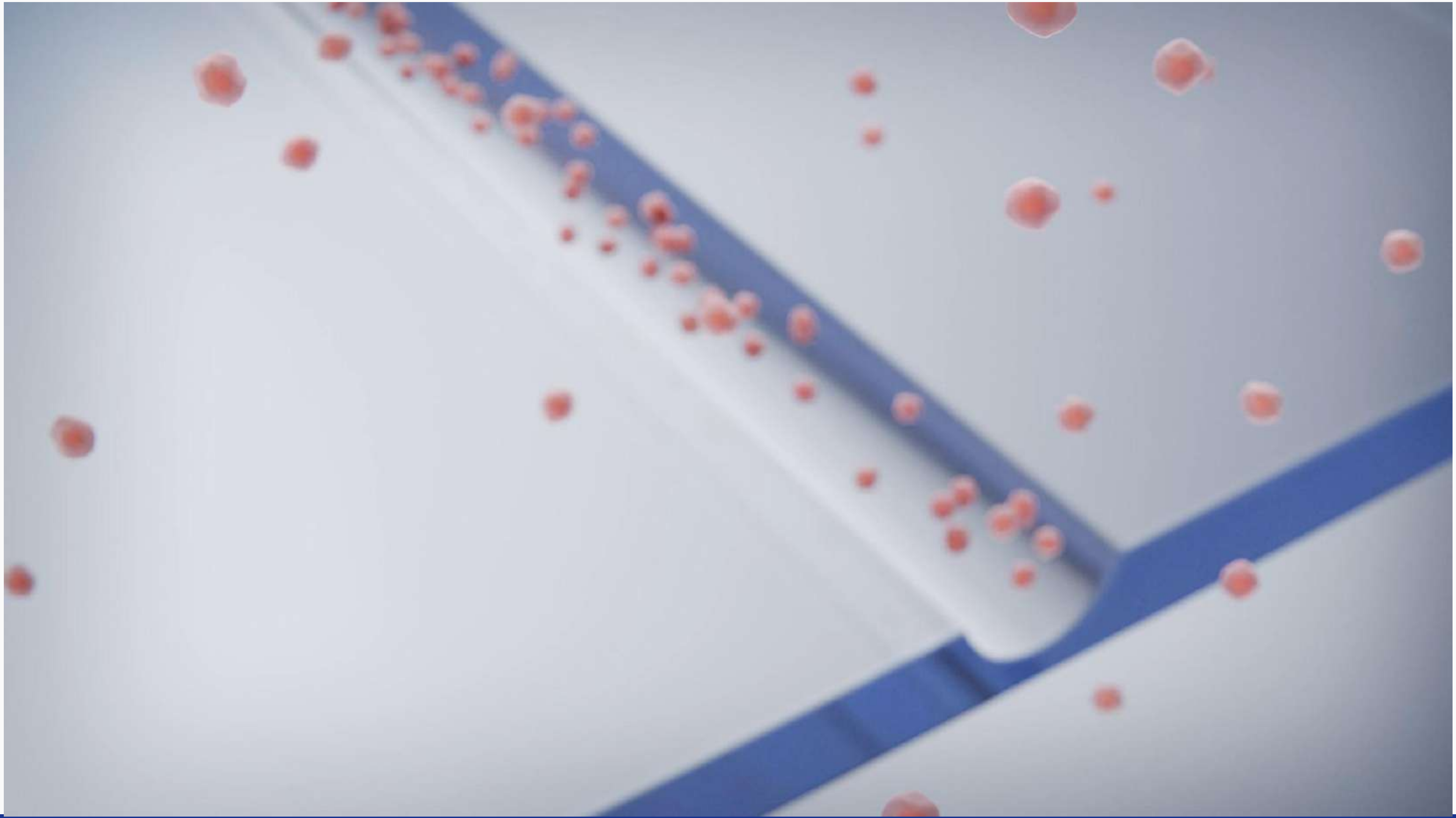
First-in-human

Autologous
iPSC-derived
RPE sheet,
2013.08-2015.09

Last 10 years needed to mature technologies (e.g. surgery, protocol)

1 patient of wAMD;

RPE strip transplantation

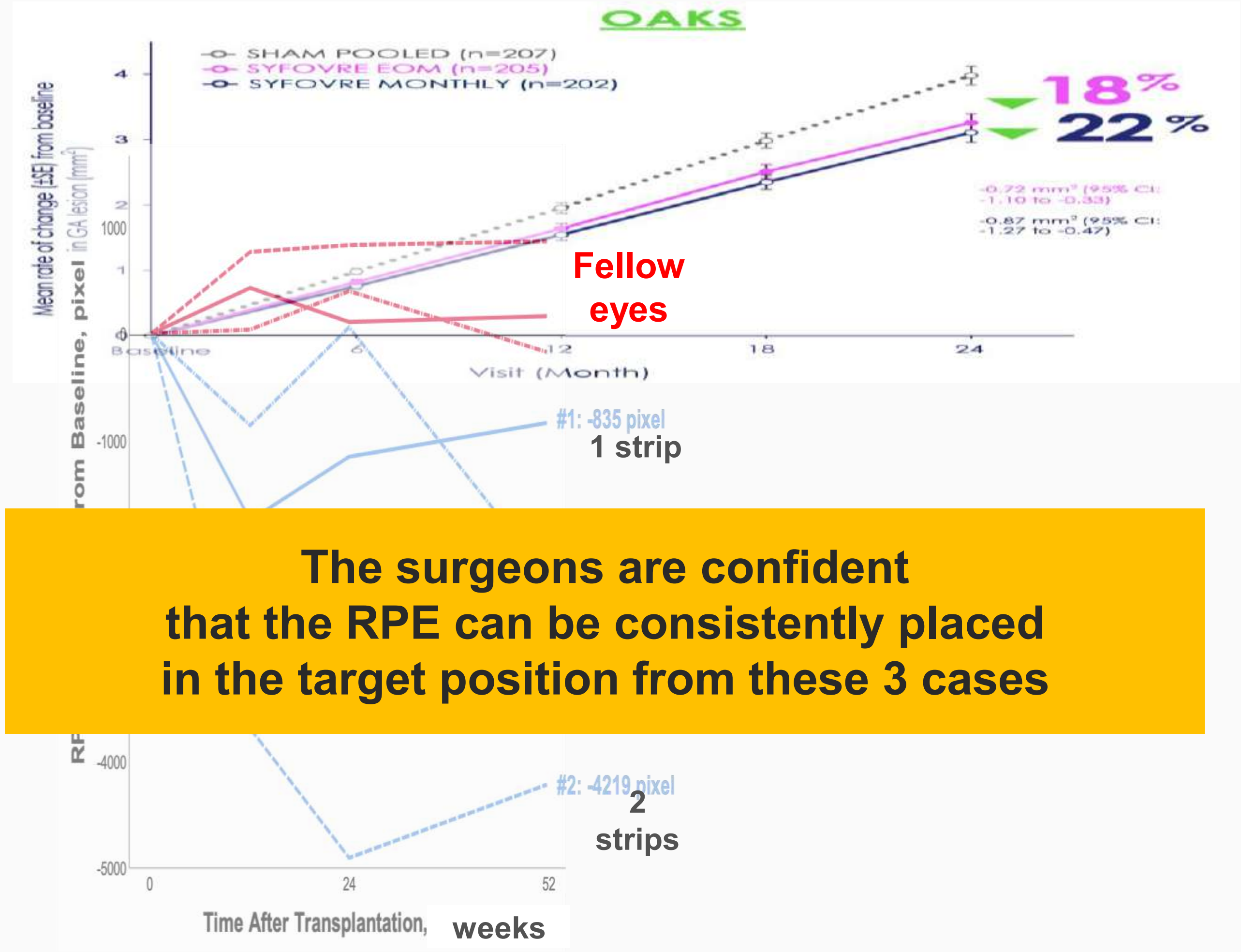


RPE strip Safety & Efficacy (n=3)

Complication

Ocular SAE	RPE strip (n=3)	RPE suspension (n = 5)
ERMembrane (removal ope)	1 (33%) 0	3 (60%) 1 (20%)
Macular edema	1 (33%)	1 (8%)
Subretinal fluid	0	0
Retinal hemorrhage	0	0
RPE detachment	0	0
Neovascularization	0	0
Retinoschysis	0	0
Retinal tear	0	0
Subretinal fibrosis	0	0
Macular hole	0	1

Red line: Most severe adverse event

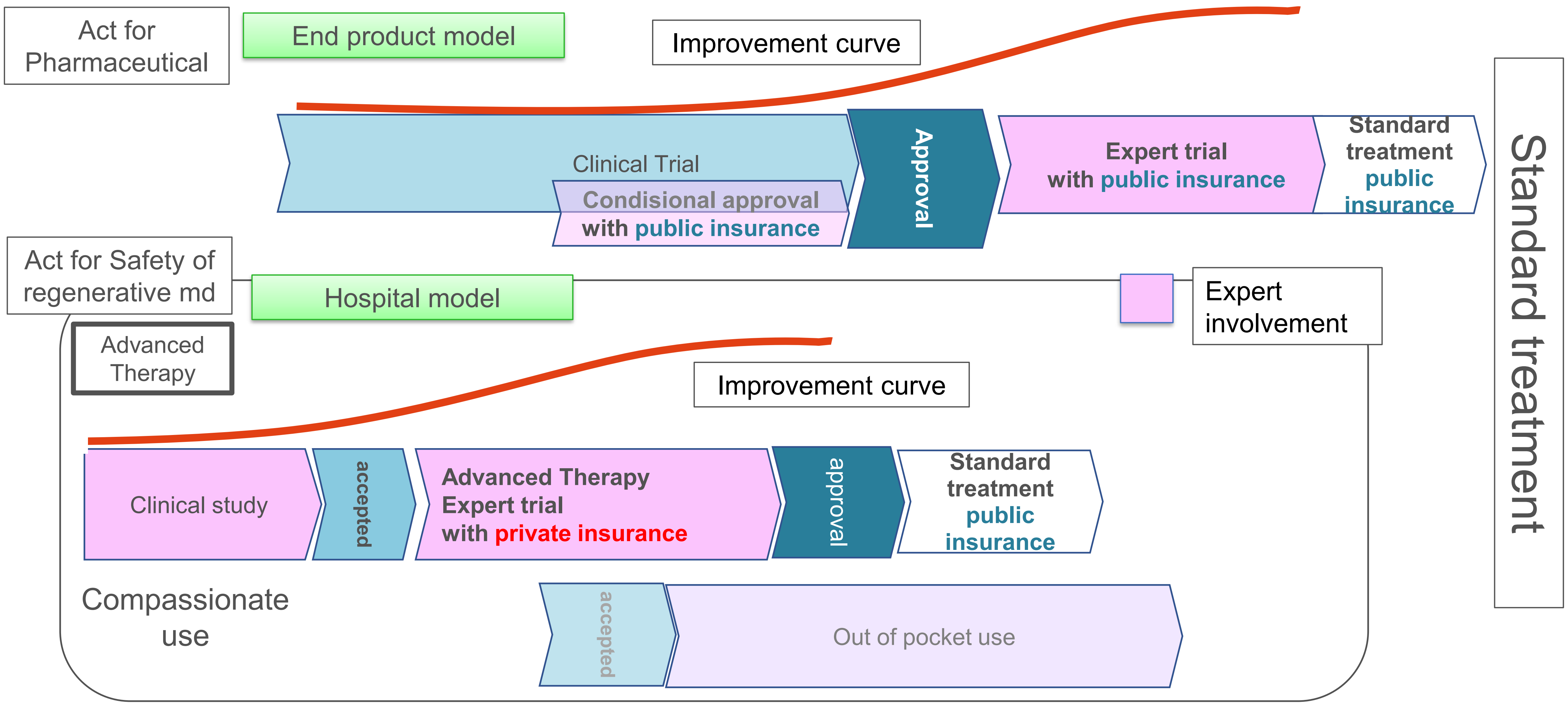


The surgeons are confident that the RPE can be consistently placed in the target position from these 3 cases

Sakai et al. Ophthalmol. Sci. 2025

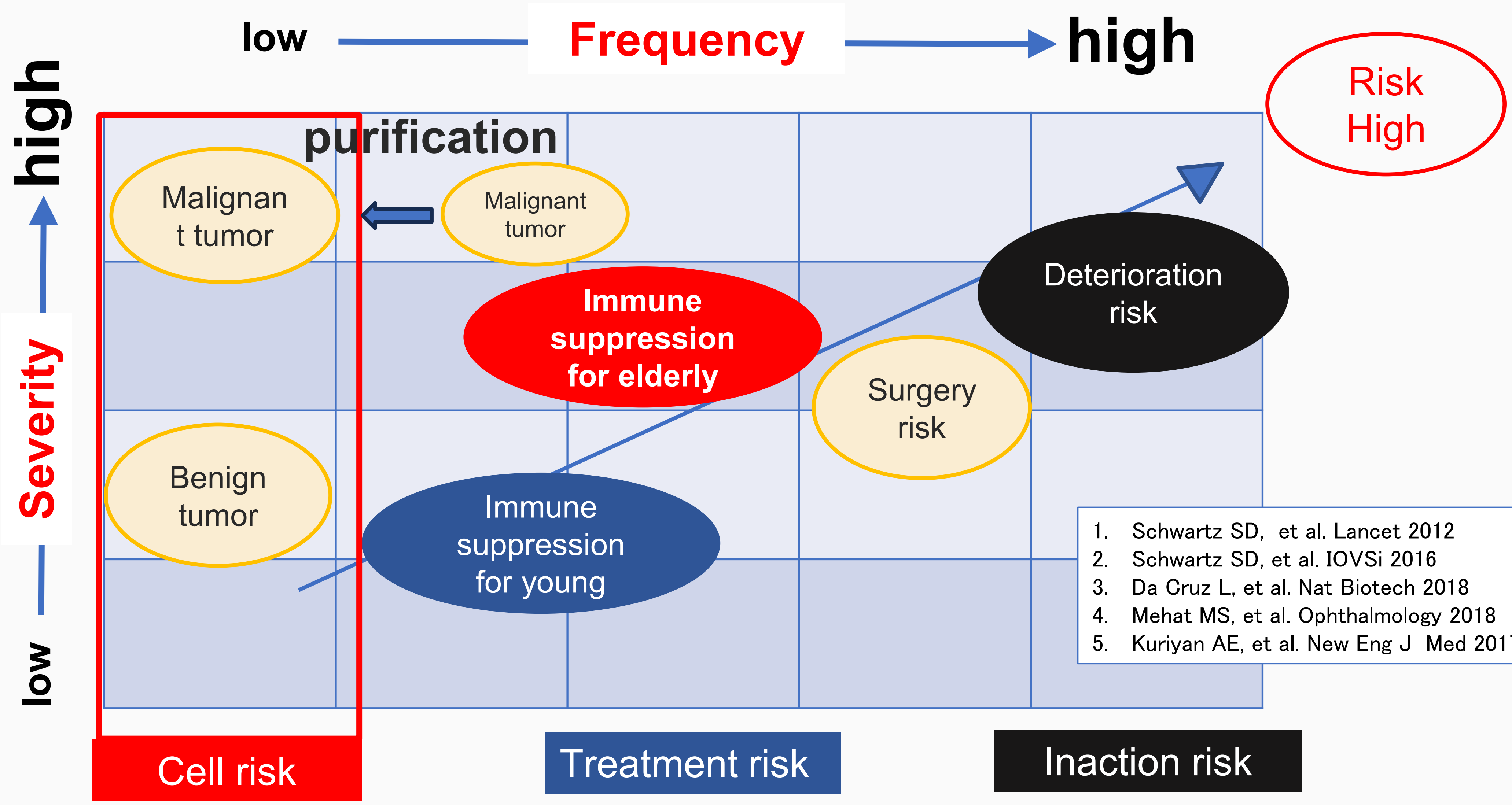
2014～

Various tracks for cell & gene therapy development in Japan



Risk Matrix

for iPSC-RPE transplantation　(Image)



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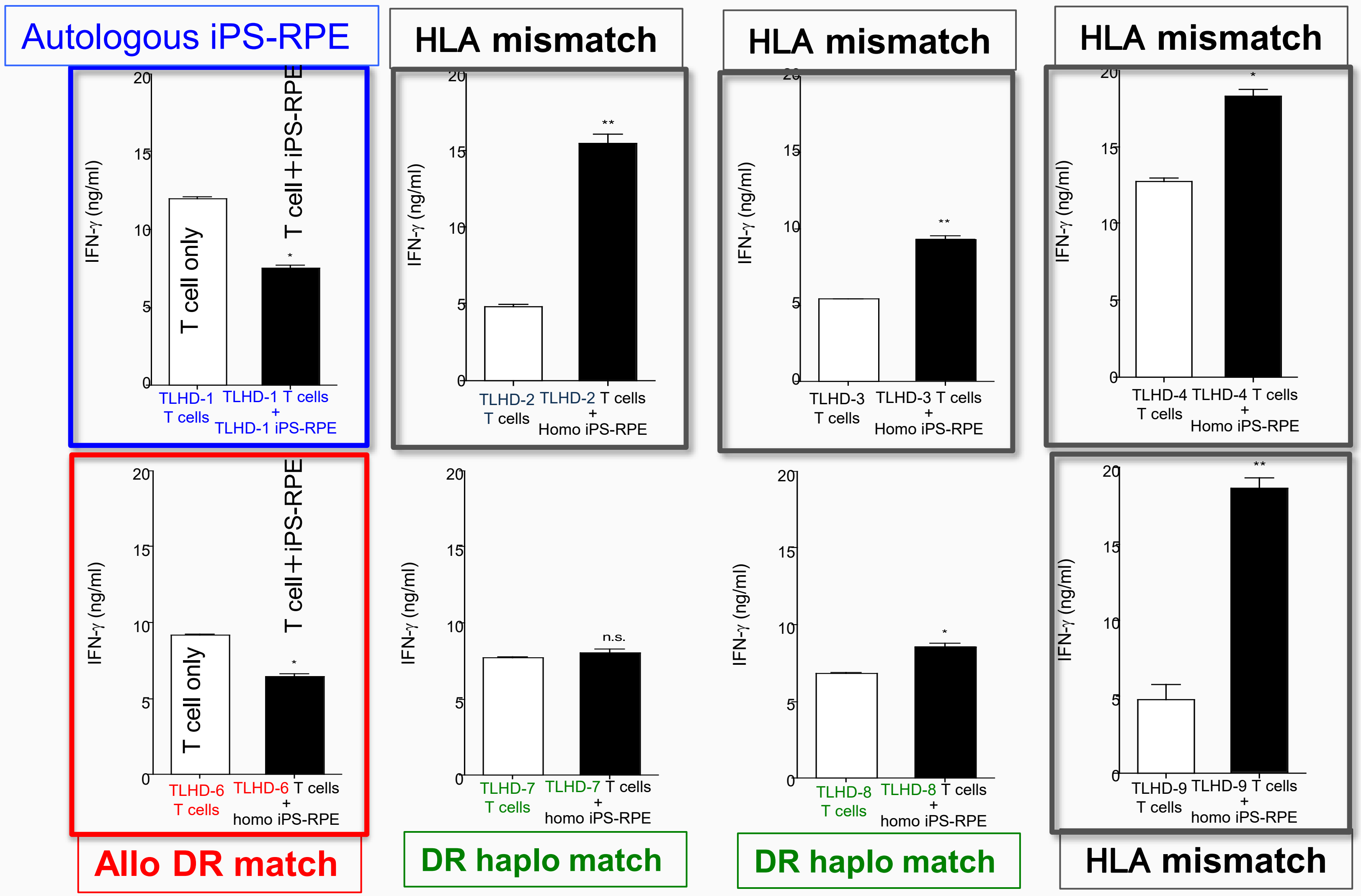
(In vitro) HLA-DR matched allogeneic iPS-RPE suppress T cell activity as well as autologous transplantation

(Sugita et al. Stem Cell Reports 2016)



T cells

TLHD-1 : DR9/DR16 Auto
TLHD-2 : DR4/DR13
TLHD-3 : DR4/DR8
TLHD-4 : DR4/-
TLHD-6 : DR8/DR15 (DRB1*15:02)
DR match
TLHD-7 : DR14/DR15 (DRB1*15:01) DR haplomatch
TLHD-8 : DR12/DR15 (DRB1*15:01) DR haplomatch
TLHD-9 : DR4/DR12

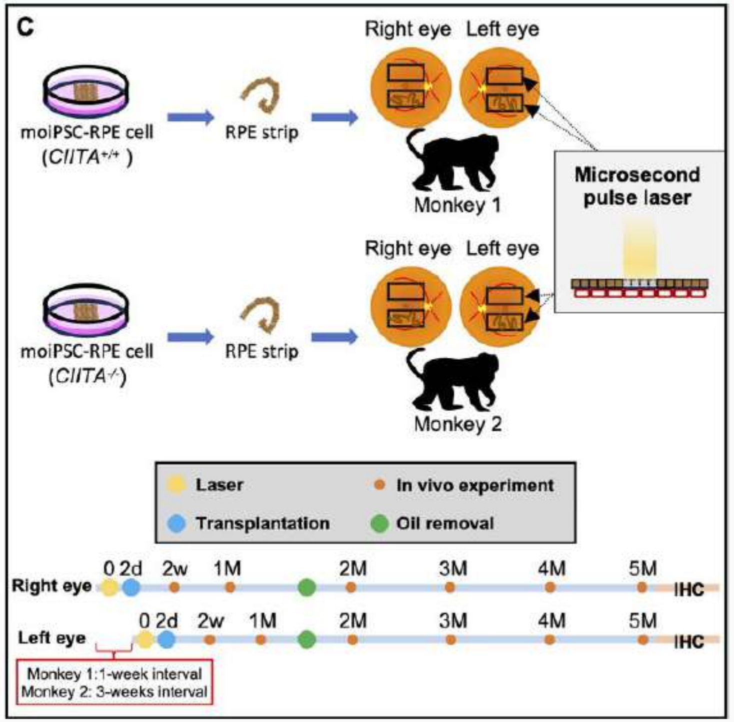
iPS

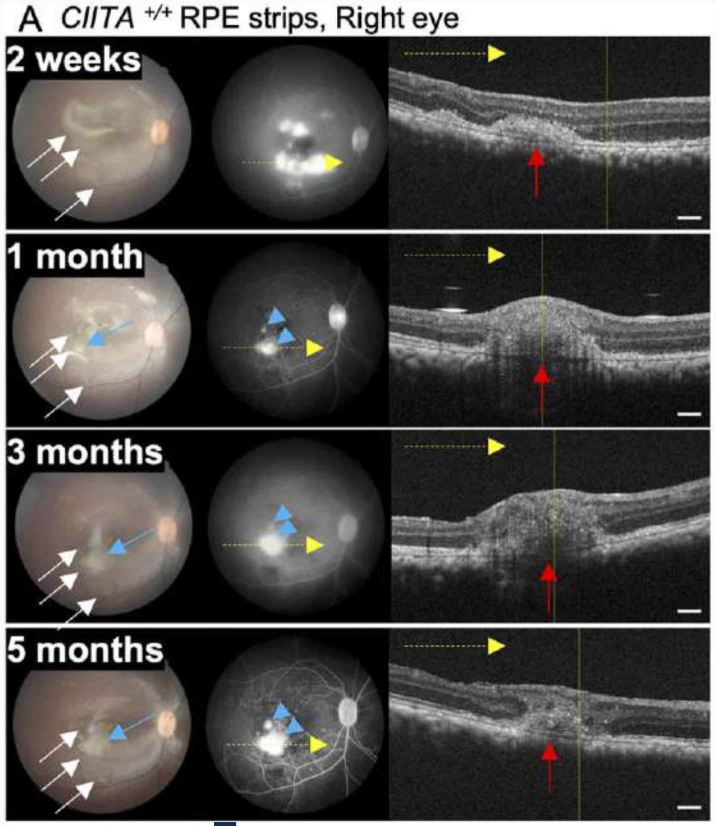
Homo (454E2) iPS: DR15/- (DRB1*15:02)

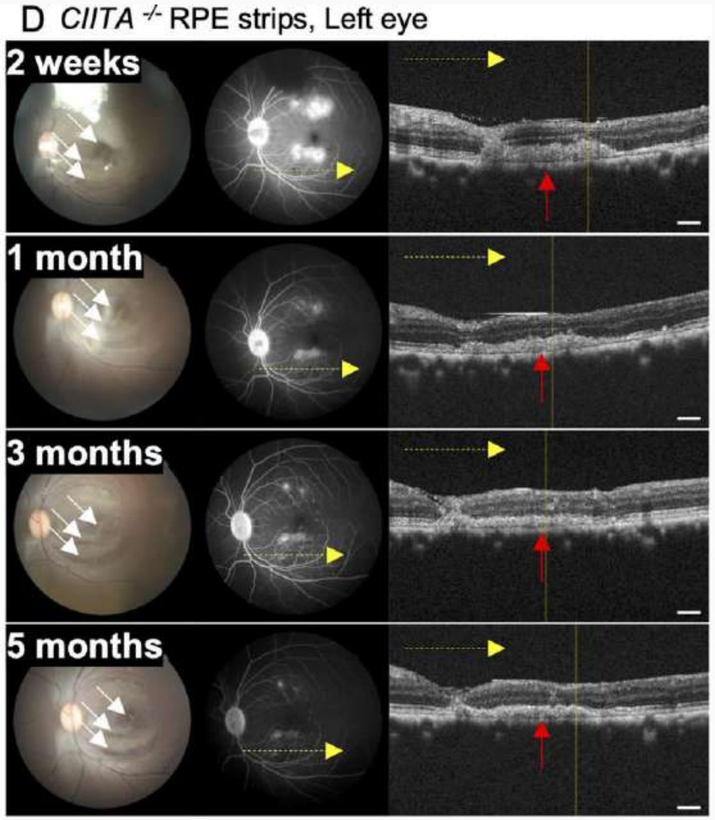
*P < 0.05, **P < 0.005
n.s. – not significant

Allogenic Transplantation of RPE Strips Lacking MHC Class II Can Avoid Rejection in Nonhuman Primate Eyes (Ozaki et al. IOVS Invest. Ophthalmol. Vis. Sci. 2025)

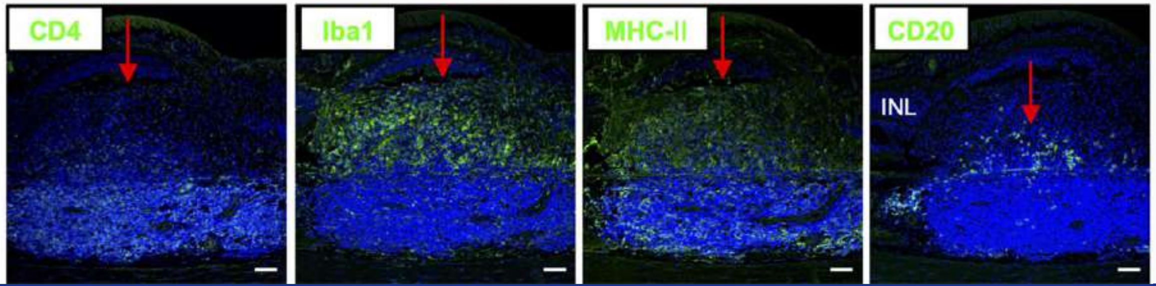
VISION CARE







***CIITA*+/+ RPE**



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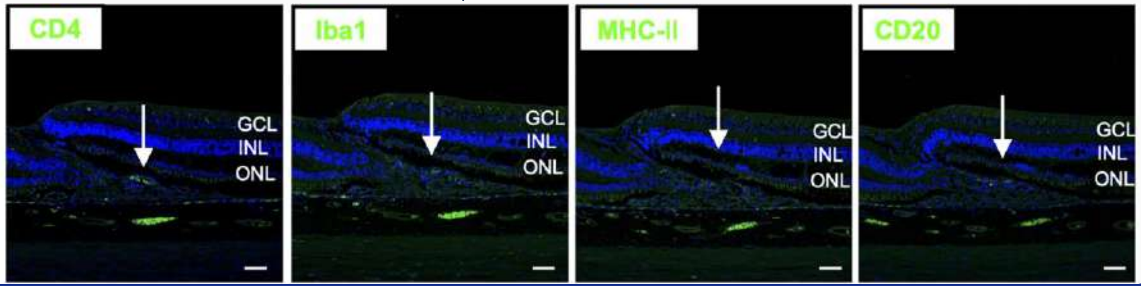


RPE& photoreceptor cells HLA expression

RPE allograft

rejected

in Monkey

	HLA class1	HLA class 2
RPE normal	+	−
RPE inflammation	+	+
Photoreceptor normal	+	−
Photoreceptor inflammation	+	−

Virus infection | Immune deficiency

RPE

For the standard treatment (2017~)
Allogeneic iPSC-RPE transplantation

Totally 10 patients
- No serious adverse event
- No severe surgical complication
- All have long survival of the grafts
- All patients maintain their visual function (efficacy)
- Two of them showed clear improvement of subjective symptoms (dAMD & RP)

HLA 6 loci match
w/o immune suppression

Final product

HLA partially KO
RPE strip
w/o immune suppression

with immune suppression

Allogenic
iPSC-derived
RPE strip
2022 - 2023

Allogenic
iPSC-derived
RPE cell suspension
2017.03-2018.09

3 patients of dAMD & RP

First-in-human

Autologous
iPSC-derived
RPE sheet,
2013.08-2015.09

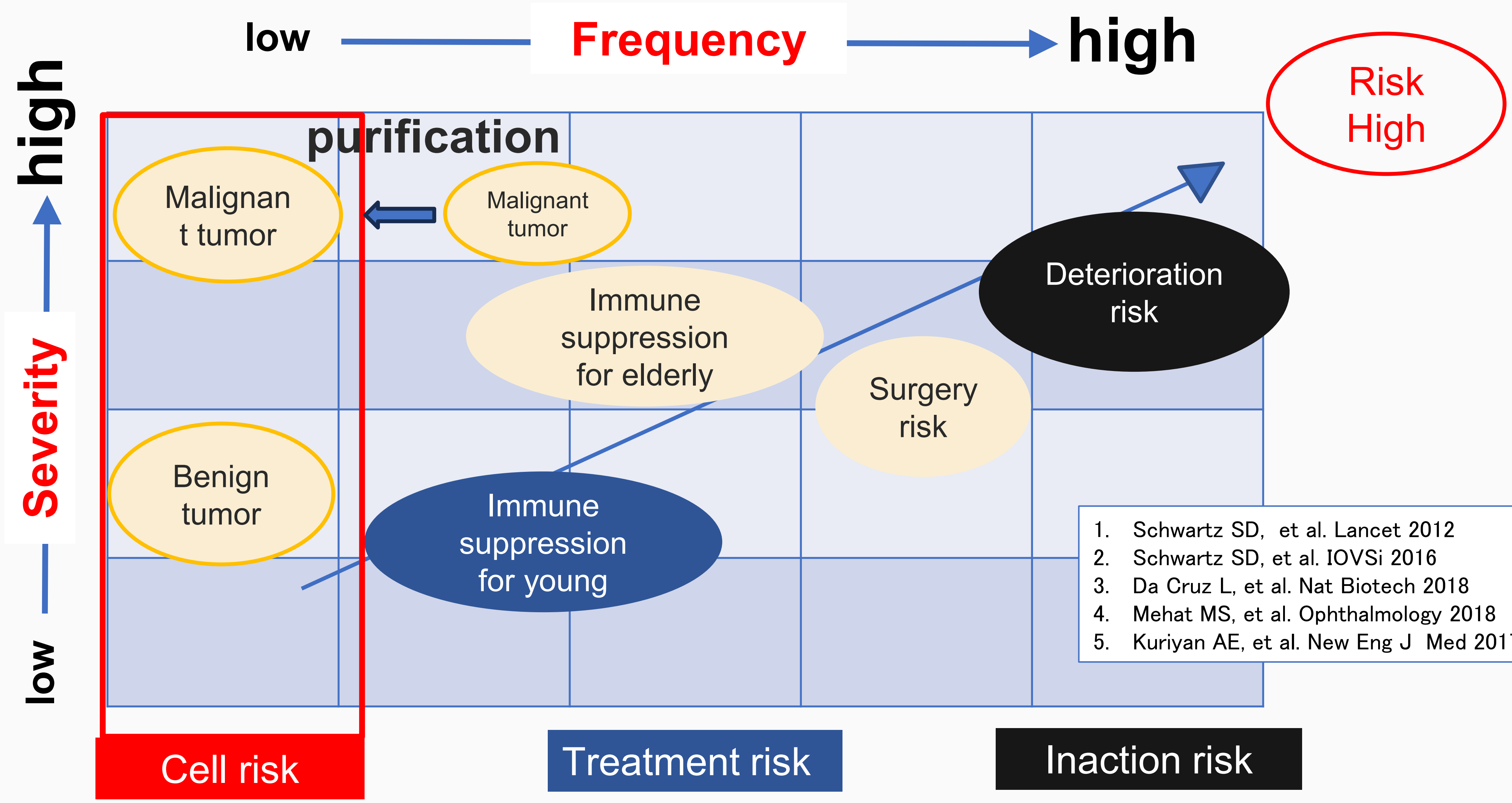
6 patients of wAMD

Last 10 years needed to mature technologies (e.g. surgery, protocol)

1 patient of wAMD;

Risk Matrix

for iPSC-RPE transplantation (Image)



1. Schwartz SD, et al. Lancet 2012
2. Schwartz SD, et al. IOVSi 2016
3. Da Cruz L, et al. Nat Biotech 2018
4. Mehat MS, et al. Ophthalmology 2018
5. Kuriyan AE, et al. New Eng J Med 2017

Difference of the Strategies

RPE

Before the 1st Case

- Determine the Mode of Action – replacement of RPE cell layer
- Determine the CQA – purity

The 2nd & 3rd clinical study

- Select the formulation to solve the risk of surgery – RPE strip
- Solve manufacturing skill transfer risks and scale up – using the humanoid robot
- Expand the surgeons and hospitals

Before a clinical trial

- The immune suppression risk – Modify the HLA

Photoreceptor

Before the 1st clinical study

- Determine the Mode of Action – replacement of photoreceptor cell layer
- Determine the formulation – retinal organoid

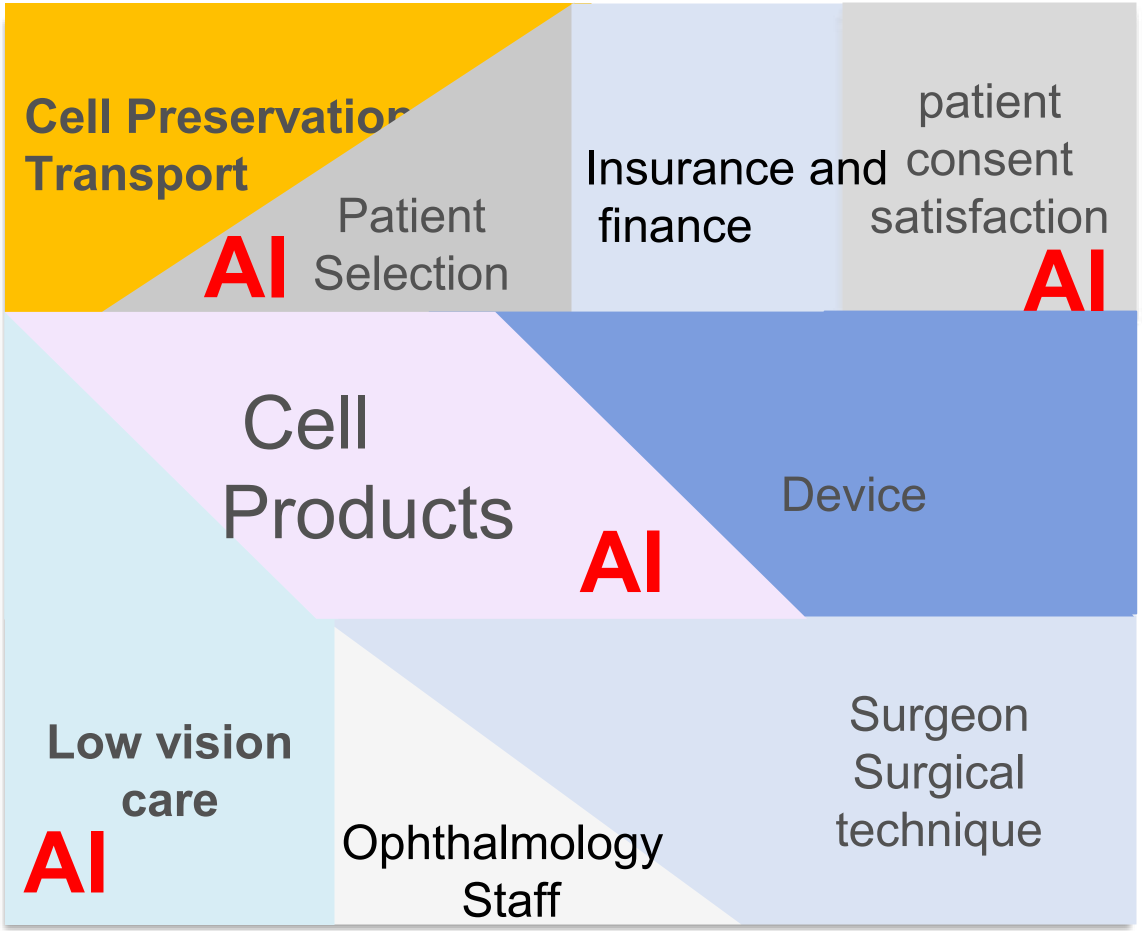
The 2nd clinical study

- Improve the efficacy – islet-1 KO iPS organoid
- Determine the CQA – qPCR, morphology
- Select the suitable iPS cell lines – animal model & electrophysiology
- Expand the target cases – RPE & photoreceptor combined treatment

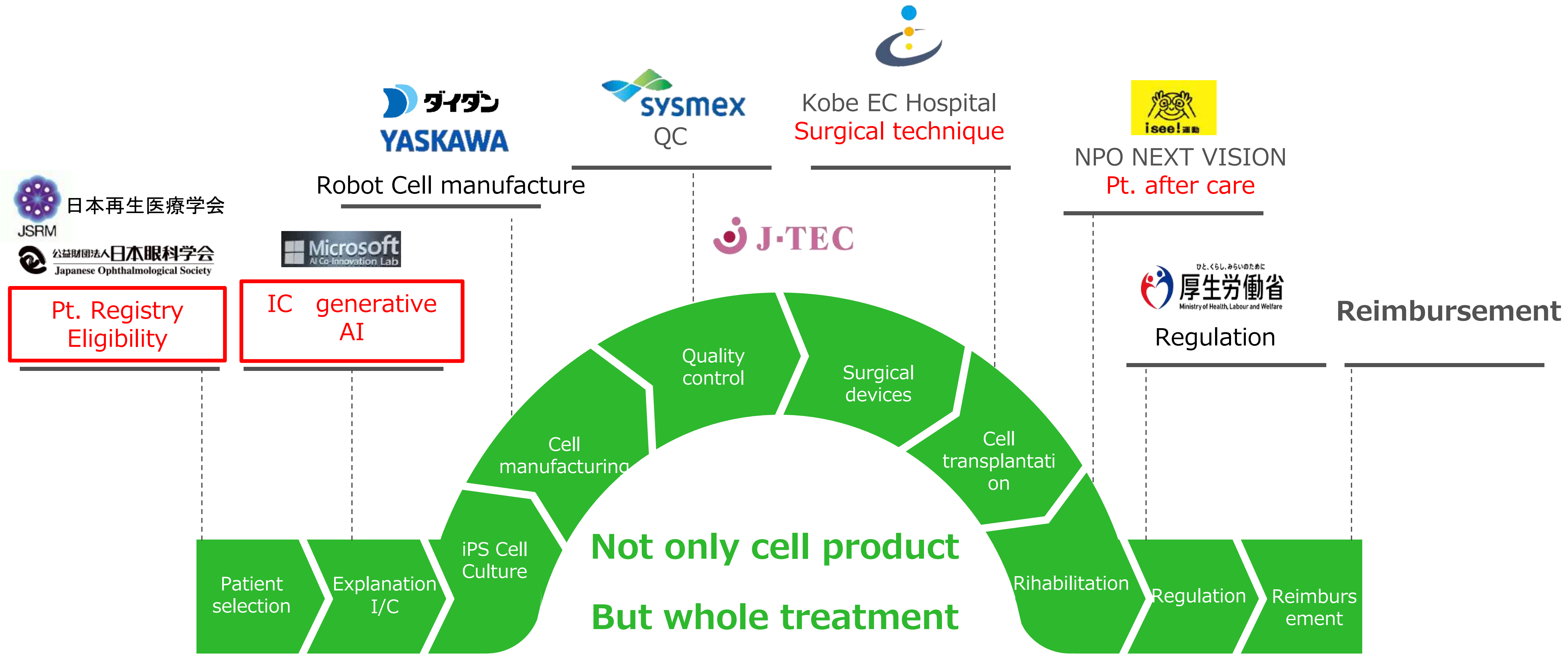
Not necessary

Start the clinical trial with the final product

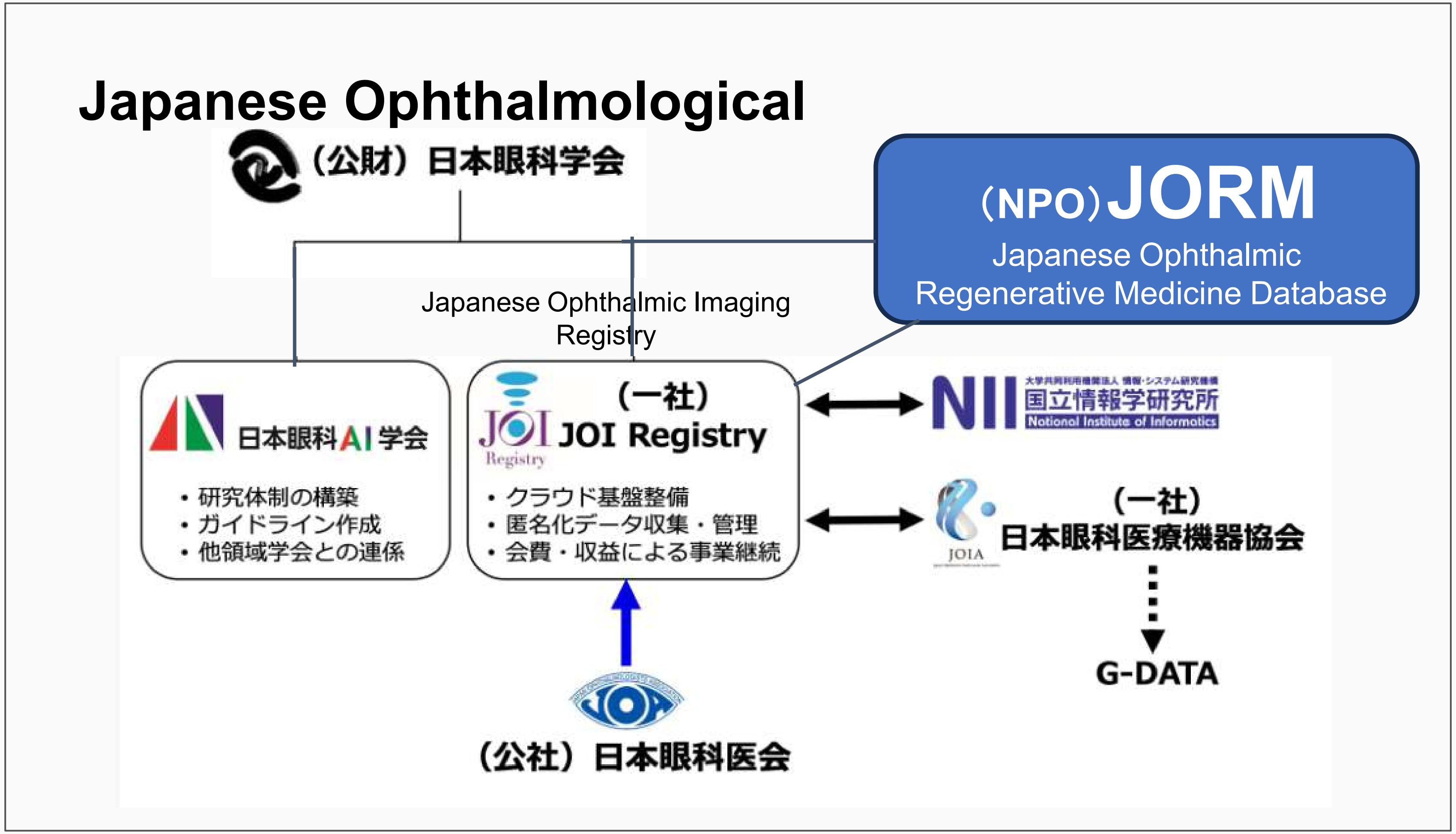
Factors in Regenerative Medicine
Product ≠ Treatment



Whole treatment Development



Patient Selection Registry
for Regenerative Medicine in Ophthalmology



Consortium for RPE transplantation

Kyoto Univ.
Kyushu Univ.
Yokohama City Univ.
Aichi Med Univ.
Hiroshima Univ.
Tokyo Univ.
Kobe Eye Center
-
-
-

More than 20 Universities

NRMD/CR ⇒ Clinical trial ⇒PMS data utilization

Japanese Association of Medical Science

日本医学会 The Japanese Association of Medical Sciences

Registry Council for Regenerative Medicine

NRMD/CR

NRMD/PMS

case data

As a historical control
Utilized for new development, indication expansion, and (re)review

clinical research

clinical trial

(with conditions and deadlines) approval

PMS

Act for Safety of Regenerative Medicine,

Pharmaceuticals and Medical Devices Law

JSRM

infrastructure improvement

Pmda

pharmaceutical consultation

Retinal Regenerative Medicine Explanatory Software

Problem now

- I can't ask questions about what I don't understand.
- Technical words are difficult.

- take time
- It needs to be explained to the patient accordingly.
- Only a physician who knows regenerative medicine well can explain it.

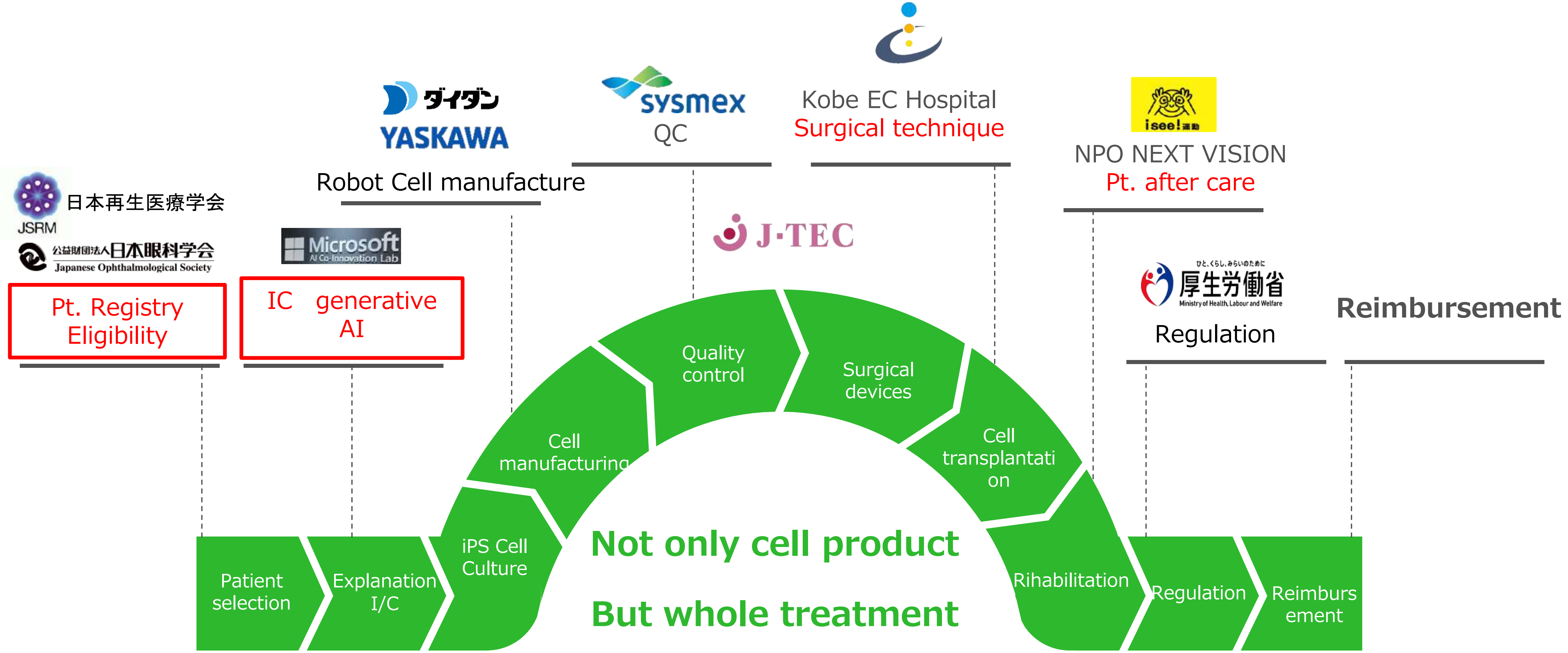
Solution

Emily

How much therapeutic effect can be~

Transplantation of RPE cells made from iPS cells is thought to be ~~

Whole treatment Development



CELL THERAPY